SALTWATER SPECIES

Selenite

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Blue mussel (embryo), *Mytilus edulis*	S, U	Selenium oxide	33.79	>10,000	>10,000	Martin et al. 1981
Bay scallop (juvenile), *Argopecten irradians*	R, U	Sodium selenite	25	255	255	Nelson et al. 1988
Pacific oyster (embryo), *Crassostrea gigas*	S, U	Selenium oxide	33.79	>10,000	-	Glickstein 1978; Martin et al. 1981
Pacific oyster (embryo), *Crassostrea gigas*	S, U	Sodium selenite	33.79	>10,000	>10,000	Glickstein 1978
Surf clam (juvenile), *Spisula solidissima*	R, U	Sodium selenite	25	1,900	1,900	Nelson et al. 1988
Copepod (adult), *Acartia clausi*	S, U	Selenious acid	30	2,110	2,110	Lussier 1986
Copepod (adult), *Acartia tonsa*	S, U	Selenious acid	30	839	839	Lussier 1986
Mysid (juvenile), *Americamysis bahia*	S, U	Selenious acid	-	600	-	U.S. EPA 1978
Mysid (juvenile), *Americamysis bahia*	F, M	Selenious acid	15-20	1,500	1,500	Ward et al. 1981
Brown shrimp (juvenile), *Penaeus aztecus*	S, U	Sodium selenite	30	1,200	1,200	Ward et al. 1981
Dungeness crab (zoea 1 larva), *Cancer magister*	S, U	Selenium oxide	33.79	1,040	1,040	Glickstein 1978
Blue crab (juvenile), *Callinectes sapidus*	S, U	Sodium selenite	30	4,600	4,600	Ward et al. 1981

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Haddock (larva), *Melanogrammus aeglefinus*	S, U	Selenious acid	30	599	599	Cardin 1986
Sheepshead minnow (juvenile), *Cyrinodon variegatus*	S, U	Selenious acid	-	6,700	-	Heitmuller et al. 1981
Sheepshead minnow (juvenile), *Cyrinodon variegatus*	F, M	Sodium selenite	30	7,400	7,400	Ward et al. 1981
Atlantic silverside (juvenile), *Menidia menidia*	S, U	Selenious acid	30	9,725	9,725	Cardin 1986
Fourspine stickleback (adult), *Apeltes quadracus*	S, U	Selenious acid	30	17,350	17,350	Cardin 1986
Striped bass, *Morone saxatilis*	S, U	Sodium selenite	1	1,550	-	Palawski et al. 1985
Striped bass (24 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	3,400	-	Chapman 1992
Striped bass (25 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	3,300	-	Chapman 1992
Striped bass (31 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	3,800	-	Chapman 1992
Striped bass (32 d posthatch), *Morone saxatilis*	S, U	Sodium selenite	5	3,900	3,036	Chapman 1992
Pinfish (juvenile), *Lagodon rhomboides*	S, U	Sodium selenite	30	4,400	4,400	Ward et al. 1981
Summer flounder (embryo), *Paralichthys dentatus*	S, U	Selenious acid	30.2	3,497	3,497	Cardin 1986
Winter flounder (larva), *Pseudopleuronectes americanus*	S, U	Selenious acid	30	14,240	-	Cardin 1986

Table 1b. Acute Toxicity of Selenium to Saltwater Animals (continued)

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Winter flounder (larva), *Pseudopleuronectes americanus*	S, U	Selenious acid	28	**15,070**	14,649	Cardin 1986

Selenate

Species	Method[a]	Chemical	Salinity (g/kg)	LC50 or EC50 (µg/L)[b]	Species Mean Acute Value (µg/L)	Reference
Striped bass (24 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	26,300[c]	-	Chapman 1992
Striped bass (25 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	23,700[c]	-	Chapman 1992
Striped bass (31 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	26,300[c]	-	Chapman 1992
Striped bass (32 d posthatch), *Morone saxatilis*	S, U	Sodium selenate	5	29,000[c]	-	Chapman 1992
Striped bass (juvenile), *Morone saxatilis*	F, M	Sodium selenate	6.0-6.5	85,840[c]	-	Klauda 1985a,b
Striped bass (prolarvae), *Morone saxatilis*	F, M	Sodium selenate	3.5-4.2	**9,790**	9,790	Klauda 1985a,b

[a] S = static; R = renewal; F = flow-through; M = measured; U = unmeasured.
[b] Concentration of selenium, not the chemical. **Note:** The values underlined in this column were used to calculate the SMAV for the respective species.
[c] Not used in calculation of Species Mean Acute Value because data are available for a more sensitive life stage.

Table 2a. Ranked Freshwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (µg/L)[b]	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		FRESHWATER SPECIES		
		Selenite		
28	203,000	Leech, *Nephelopsis obscura*	203,000	1
27	42,500	Midge, *Tanytarsus dissimilis*	42,500	1
26	35,356	Midge, *Chironomus decorus*	48,200	1
		Midge, *Chironomus plumosus*	25,934	2
25	35,000	Common carp, *Cyprinus carpio*	35,000	1
24	34,914	Snail, *Aplexa hypnorum*	34,914	2
23	28,500	Bluegill, *Lepomis macrochirus*	28,500	1
22	26,100	Goldfish, *Carassius auratus*	26,100	1
21	24,100	Snail, *Physa sp.*	24,100	1
20	24,008	White sucker, *Catostomus commersoni*	30,176	2
		Flannelmouth sucker *Catostomus latipinnis*	19,100	1
19	15,675	Arctic grayling *Thymallus arcticus*	15,675	1
18	13,600	Channel catfish, *Ictalurus punctatus*	13,600	1
17	12,801	Colorado squawfish, *Ptychocheilus lucias*	12,801	6
16	12,600	Mosquitofish, *Gambusia affinis*	12,600	1
15	11,700	Yellow perch, *Perca flavescens*	11,700	1
14	11,200	Golden shiner, *Notemigonus crysoleucas*	11,200	1

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
13	10,580	Chinook salmon, *Oncorhynchus tshawytscha*	15,596	6
		Coho salmon, *Oncorhynchus kisutch*	7,240	3
		Rainbow trout, *Oncorhynchus mykiss*	10,488	2
12	10,200	Brook trout *Salvelinus fontinalis*	10,200	1
11	9,708	Bonytail *Gilas elegans*	9,708	5
10	7,710	Worm, *Tubifex tubifex*	7,710	1
9	7,679	Razorback sucker, *Xyrauchen texanus*	7,679	6
8	6,500	Flagfish, *Jordanella floridae*	6,500	1
7	3,489	Amphipod, *Gammarus pseudolimnaeus*	3,489	5
6	2,209	Fathead minnow, *Pimephales promelas*	2,209	8
5	1,783	Striped bass, *Morone saxatilis*	1,783	2
4	1,700	Hydra, *Hydra sp.*	1,700	1
3	1,341	Cladoceran, *Daphnia magna*	905.3	11
		Cladoceran, *Daphnia pulex*	1,987	1
2	<515.3	Cladoceran, *Ceriodaphnia affinis*	<603.6	4
		Cladoceran, *Ceriodaphnia dubia*	440	1
1	461.4	Amphipod, *Hyalella azteca*	461.4	5

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		Selenate		
18	442,000	Leech, *Nephelopsis obscura*	442,000	1
17	193,000	Snail, *Aplexa hypnorum*	193,000	1
16	66,000	Channel catfish, *Ictalurus punctatus*	66,000	1
15	63,000	Bluegill, *Lepomis macrochirus*	63,000	1
14	56,493	Chinook salmon, *Oncorhynchus tshawytscha*	112,918	5
		Coho salmon, *Oncorhynchus kisutch*	33,972	3
		Rainbow trout, *Oncorhynchus mykiss*	47,000	1
13	56,081	Arctic grayling, *Thymallus arcticus*	56,081	2
12	53,454	Colorado squawfish, *Ptychocheilus lucius*	53,454	6
11	37,586	Bonytail, *Gila elegans*	37,586	5
10	26,900	Flannelmouth sucker *Catostomus latipinnis*	26,900	1
9	23,700	Midge, *Chironomus decorus*	23,700	1
8	20,000	Midge, *Paratanytarsus parthenogeneticus*	20,000	1
7	13,211	Razorback sucker, *Xyrauchen texanus*	13,211	6
6	12,282	Fathead minnow, *Pimephales promelas*	12,282	5
5	7,300	Hydra, *Hydra sp.*	7,300	1
4	2,741	Amphipod, *Gammarus lacustris*	3,054	1

Table 2a. Ranked Freshwater Genus Mean Acute Values (continued)

Rank[a]	Genus Mean Acute Value (μg/L)	Species	Species Mean Acute Value (μg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		Amphipod, *Gammarus pseudolimnaeus*	2,460	5
3	2,073	Amphipod, *Hyalella azteca*	2,073	4
2	926.8	Cladoceran, *Daphnia magna*	2,118	6
		Cladoceran, *Daphnia pulex*	1,528	1
		Cladoceran, *Daphnia pulicaria*	246	1
1	376	Cladoceran, *Ceriodaphnia dubia*	376	1

[a.] Ranked from most resistant to most sensitive based on Genus Mean Acute Value. Inclusion of "greater than" and "less than" values does not necessarily imply a true ranking, but does allow use of all genera for which data are available so that the Final Acute Value is not unnecessarily lowered.
[b] From Table 1a.

Table 2b. Ranked Saltwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (μg/L)	Species	Species Mean Acute Value (μg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
		SALTWATER SPECIES		
		Selenite		
17	17,350	Fourspine stickleback, *Apeltes quadracus*	17,350	1
16	14,649	Winter flounder, *Pseudopleuronectes americanus*	14,649	2
15	>10,000	Blue mussel, *Mytilus edulis*	>10,000	1
14	>10,000	Pacific oyster, *Crassostrea gigas*	>10,000	2
13	9,725	Atlantic silverside, *Menidia menidia*	9,725	1
12	7,400	Sheepshead minnow, *Cyprinodon variegatus*	7,400	1
11	4,600	Blue crab, *Callinectes sapidus*	4,600	1
10	4,400	Pinfish, *Lagodon rhomboides*	4,400	1
9	3,497	Summer flounder, *Paralichthys dentatus*	3,497	1
8	3,036	Striped bass, *Morone saxatilis*	3,036	5
7	1,900	Surf clam, *Spisula solidissima*	1,900	1
6	1,500	Mysid, *Americamysis bahia*	1,500	1
5	1,331	Copepod, *Acartia clausi*	2,110	1
		Copepod, *Acartia tonsa*	839	1
4	1,200	Brown shrimp, *Penaeus aztecus*	1,200	1
3	1,040	Dungeness crab, *Cancer magister*	1,040	1
2	599	Haddock, *Melanogrammus aeglefinus*	599	1

Table 2b. Ranked Saltwater Genus Mean Acute Values

Rank[a]	Genus Mean Acute Value (µg/L)	Species	Species Mean Acute Value (µg/L)[b]	Number of Acute Values used to Calculate Species Mean Value[b]
1	255	Bay scallop, *Argopecten irradians*	255	1
		Selenate		
1	9,790	Striped bass, *Morone saxatilis*	9,790	1

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value. Inclusion of "greater than" and "less than" values does not necessarily imply a true ranking, but does allow use of all genera for which data are available so that the Final Acute Value is not unnecessarily lowered.
[b] From Table 1b.

Selenite

  Fresh water

  Final Acute Value = 514.9 µg/L

  Criterion Maximum Concentration = (514.9 µg/L)/2 = 257.5 µg/L

  Salt water

  Final Acute Value = 253.4 µg/L

  Criterion Maximum Concentration = (253.4 µg/L)/2 = 126.7 µg/L

Selenate

  Fresh water

  Final Acute Value = 369.6 µg/L

  Criterion Maximum Concentration = (369.6 µg/L)/2 = 184.8 µg/L

Table 3a. Ratios of Freshwater Species Mean Acute Values for Selenite and Selenate.

Selenite Sensitivity Rank from Table 2a[a]	Species	Selenite Species Mean Acute Value (µg/L)[b]	Selenate Species Mean Acute Value (µg/L)[b]	Ratio
	FRESHWATER SPECIES			
28	Leech, *Nephelopsis obscura*	203,000	442,000	0.459
27	Midge, *Tanytarsus dissimilis*	42,500	NA[c]	NA
26	Midge, *Chironomus decorus*	48,200	23,700	2.033
	Midge, *Chironomus plumosus*	25,934	NA	NA
25	Common carp, *Cyprinus carpio*	35,000	NA	NA
24	Snail, *Aplexa hypnorum*	34,914	193,000	0.181
23	Bluegill, *Lepomis macrochirus*	28,500	63,000	0.452
22	Goldfish, *Carassius auratus*	26,100	NA	NA
21	Snail, *Physa sp.*	24,100	NA	NA
20	White sucker, *Catostomus commersoni*	30,176	NA	NA
	Flannelmouth sucker *Catostomus latipinnis*	19,100	26,900	0.710
19	Arctic grayling *Thymallus articus*	15,675	56,081	0.280
18	Channel catfish, *Ictalurus punctatus*	13,600	66,000	0.206
17	Colorado squawfish, *Ptychocheilus lucias*	12,801	53,454	0.239
16	Mosquitofish, *Gambusia affinis*	12,600	NA	NA
15	Yellow perch, *Perca flavescens*	11,700	NA	NA
14	Golden shiner, *Notoemigonus crysoleucas*	11,200	NA	NA
13	Chinook salmon, *Oncorhynchus tshawytscha*	15,596	112,948	0.138

Table 3a. Ratios of Freshwater Species Mean Acute Values for Selenite and Selenate (continued).

Selenite Sensitivity Rank from Table 2a[a]	Species	Selenite Species Mean Acute Value $(\mu g/L)^b$	Selenate Species Mean Acute Value $(\mu g/L)^b$	Ratio
	Coho salmon, *Oncorhynchus kisutch*	7,240	33,972	0.213
	Rainbow trout, *Oncorhynchus mykiss*	10,488	47,000	0.223
12	Brook trout *Salvelinus fontinalis*	10,200	NA	NA
11	Bonytail *Gilas elegans*	9,708	37,586	0.258
10	Worm, *Tubifex tubifex*	7,710	NA	NA
9	Razorback sucker, *Xyrauchen texanus*	7,679	13,211	0.581
8	Flagfish, *Jordanella floridae*	6,500	NA	NA
7	Amphipod, *Gammarus pseudolimnaeus*	3,489	2,460	1.418
6	Fathead minnow, *Pimephales promelas*	2,209	12,282	0.180
5	Striped bass, *Morone saxatilis*	1,783	NA	NA
4	Hydra, *Hydra sp.*	1,700	7,300	0.233
3	Cladoceran, *Daphnia magna*	905.3	2,118	0.427
	Cladoceran, *Daphnia pulex*	1,987	1,528	1.300
2	Cladoceran, *Ceriodaphnia affinis*	<603.6	NA	NA
	Cladoceran, *Ceriodaphnia dubia*	440	376	1.170
1	Amphipod, *Hyalella azteca*	461.4	2,073	0.223

[a] Ranked from most resistant to most sensitive based on selenite Genus Mean Acute Value (from Table 2a).
[b] From Table 1a.
[c] NA = Not Available

Table 3b. Ratios of Saltwater Species Mean Acute Values for Selenite and Selenate.

Sensitivity Rank from Table 2b[a]	Species	Selenite Species Mean Acute Value $(\mu g/L)^b$	Selenate Species Mean Acute Value $(\mu g/L)^b$	Ratio
	SALTWATER SPECIES			
8	Striped bass, *Morone saxatilis*	3,036	9,790	0.310

[a] Ranked from most resistant to most sensitive based on Genus Mean Acute Value (from Table 2b).
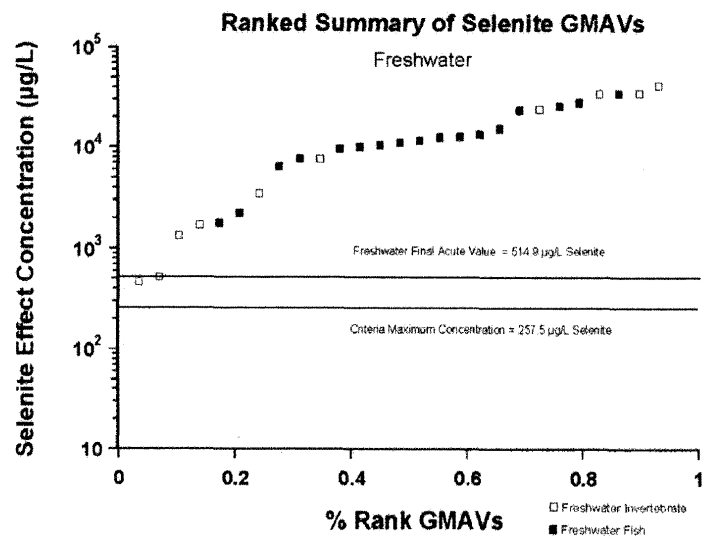[b] From Table 1b.

Figure 1.   Ranked summary of selenite GMAVs (freshwater).
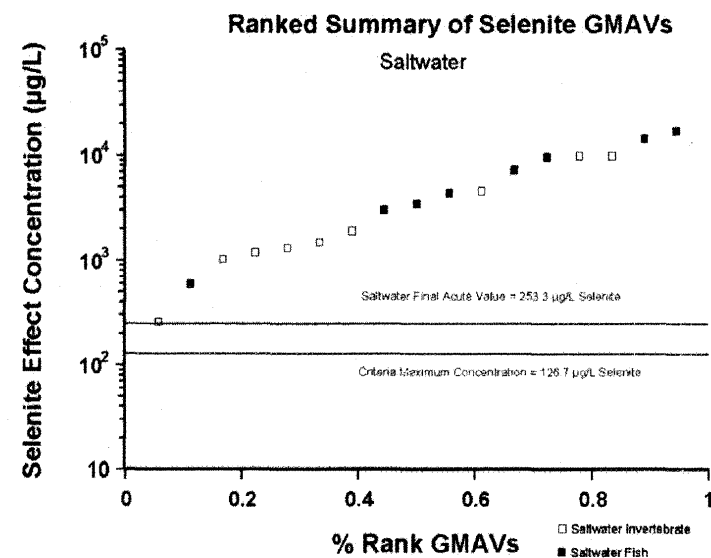

Figure 2.   Ranked summary of selenate GMAVs (saltwater).

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Ranked Summary of Selenate GMAVs
Freshwater

Freshwater Final Acute Value = 362.6 µg/L Selenate

Criteria Maximum Concentration = 184.8 µg/L Selenate

% Rank GMAVs

□ Freshwater Invertebrate
■ Freshwater Fish

Figure 3.   Ranked summary of selenate GMAVs (freshwater).

Review and Analysis of Chronic Data

Since the issuance of the 1987 chronic criterion of 5 µg/L, considerable information has come forth regarding the route of exposure of selenium to aquatic organisms. Studies have shown that diet is the primary route of exposure that controls chronic toxicity to fish, the group considered to be the most sensitive to selenium (Coyle et al. 1993; Hamilton et al. 1990; Hermanutz et al. 1996). Chronic tests in which test organisms were exposed to selenium only through water and which have measured selenium in the tissue of the test species have produced questionably low chronic values based on the tissue concentrations. Some of these water-only exposures have required aqueous concentrations of selenium of greater than 300 µg/L to attain body burdens sufficient to achieve a chronic response that would have been reached in the real world at aqueous concentrations approximately 30 times lower (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978).

Because diet controls selenium chronic toxicity in the environment and water-only exposures require unrealistic aqueous concentrations in order to elicit a chronic response, only studies in which test organisms were exposed to selenium in their diet alone or in their diet and water were considered in the derivation of a chronic value. To be able to use the chronic study results, the measurements had to include selenium in the test species tissue. Both laboratory and field studies were considered in the review process. Chronic studies reviewed were obtained through a literature search extending back to the last revision review, from information supplied to U.S. EPA through the Notice of Data Availability, and using the references cited in previous selenium criteria documents.

Selection of Medium for Expressing Chronic Criterion

Whole-body tissue concentration of selenium on a dry weight basis, for species eliciting the chronic response, was selected as the medium from which to base the chronic criterion value. As discussed above, a water-based criterion is not appropriate for selenium because diet being the most important route of exposure for chronic toxicity. The option of basing the chronic criterion on the concentration of selenium in prey species (that is, in the diet of the target species), was considered inappropriate for two reasons: 1) the concentration of selenium in the diet is an indirect measure of effects observed in the test species and is dependent on feeding behavior of the target species, and 2) selection of what organism to sample to assess attainment of a criterion based on diet is problematic in the implementation of such a criterion. Sediment has also been proposed as a medium upon which to base the selenium chronic criterion (Canton and Van Derveer 1997; Van Derveer and Canton 1997), but because of the patchiness of selenium in sediment and an insufficient amount of data to support a causal link between
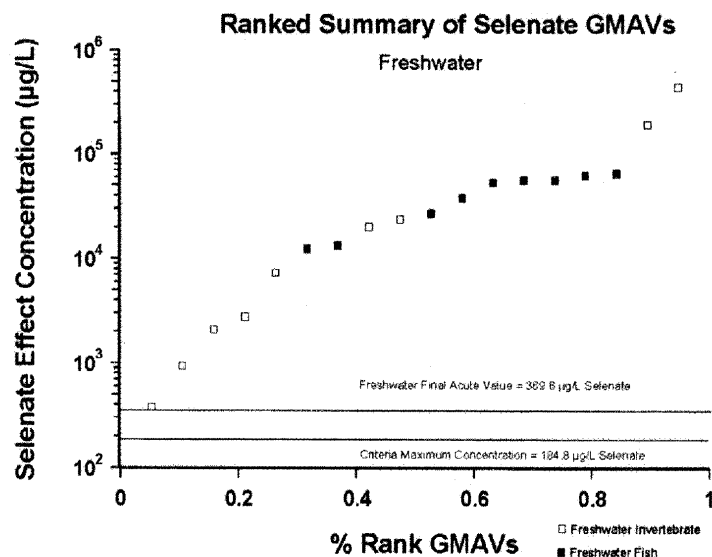
concentrations of selenium in sediment and chronic effects observed in fish (see Hamilton and Lemly 1999, for a review), a sediment-based criterion was not selected.

Besides being a direct link to chronic endpoints, a tissue-based criterion has the positive attributes of integrating many site-specific factors, such as chemical speciation and rates of transformation, large variations in temporal concentrations in water, types of organisms constituting the food chain, and rates of exchange between water, sediment, and organisms (Hamilton, in preparation; U.S. EPA 1998). Whole-body tissue was selected over specific tissue types, such as ovary, liver, kidney or muscle because of practical reasons of sampling and because a sufficient data base containing chronic effects based on whole-body tissue is present in the literature. Ovaries may be the best tissue to link selenium to chronic effects because of its role in the maternal transfer of selenium to eggs, and embryo-larval development being the most sensitive endpoint for chronic effects. However, ovarian tissue is also only available seasonally and sometimes difficult to extract in quantities sufficient for analysis, especially in smaller fish species. Whole-body larval tissue is also not practical due to sampling and seasonal constraints.

To increase the number of studies in which chronic effects could be compared with selenium concentrations in whole-body tissue, the relationship between selenium in whole-body was compared with ovary, liver and muscle tissues. Data from 12 studies that sampled whole-body as well as muscles, ovary, or liver allowed the projection of whole-body concentrations as a positive, linear function of concentrations in these individual tissues. It was not possible to estimate such relationship for kidneys and carcass because of insufficient data. Three species (rainbow trout, bluegill sunfish and largemouth bass) comprised over 95 percent of the data evaluated for these relationships.

Projections of whole-body concentrations of selenium as a linear function of concentrations of this element in muscles or ovaries appeared to be reliable (Figure 4; Appendix G; r^2 values of 0.92 and 0.84, respectively; $P < 0.01$ for both tests). Estimates from selenium concentrations in liver were not as precise ($r^2 = 0.61$), but the relationship was still highly significant ($P < 0.01$). Where appropriate, whole-body selenium concentrations were estimated from selenium concentrations in muscle, ovary and liver according to the following equations:
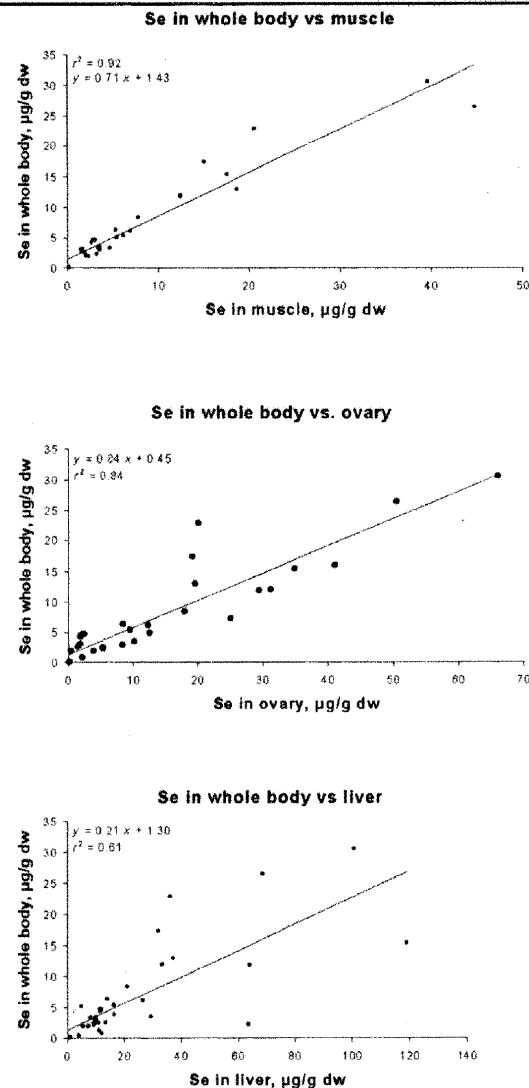

Figure 4.   Linear regressions of selenium concentrations in all tissues (whole body) against concentrations in muscle, ovary and liver tissues. Data include multiple species of fish.

$$[Se_{whole\text{-}body}] = 0.71([Se_{muscle}]) + 1.43 \qquad (I)$$

$$[Se_{whole\text{-}body}] = 0.84([Se_{ovary}]) + 0.45 \qquad (II)$$

$$[Se_{whole\text{-}body}] = 0.21([Se_{liver}]) + 1.30 \qquad (III)$$

Chronic studies that reported selenium concentrations in tissues based on wet weight were converted to dry weight using a moisture content of 0.80 (U.S. EPA 1985b).

Calculation of Chronic Values

In aquatic toxicity tests, chronic values are usually defined as the geometric mean of the highest concentration of a toxic substance at which no adverse effect is observed (highest no observed adverse effect concentration, NOAEC) and the lowest concentration of the toxic substance that causes an adverse effect (lowest observed adverse effect concentration, LOAEC). The significance of observed effects is determined by statistical tests comparing responses of organisms exposed to natural concentrations of the toxic substance (control) against responses of organisms exposed to elevated concentrations. Analysis of variance is the most common test employed for such comparisons. This approach however, has its limitations. Since neither NOAEC or LOAEC are known in advance and the number of concentrations that can be tested is constrained by logistic and financial resources, observed effects of elevated concentrations may not permit accurate estimates of chronic values. For instance, if all elevated concentrations had high adverse effects or if the difference in concentrations between two significantly different treatments was large, it would not be possible to define either the NOAEC or LOAEC with precision. Furthermore, as the concentration of some substances (e.g., selenium) naturally varies among ecosystems, a concentration that is above the normal range at one site, maybe within the normal range at a different location. In this approach to calculate chronic values, natural variation in concentrations of a substance implies that controls are site specific, and thus multiple tests are needed to define the chronic value at different locations.

An alternative approach to calculate chronic values focuses on the use of regression analysis to define the dose-response relationship. With a regression equation, which defines the level of adverse effects as a function of increasing concentrations of the toxic substance, it is possible to determine the concentration

that causes a relatively small effect, for example a 5 to 30 percent reduction in response. A reduction of 20 percent in the response observed at control (EC_{20}) was used as the chronic value because it represents a low level of effect that is generally significantly different from the control (U.S. EPA 1999). Smaller reductions in growth, survival, or other endpoints only rarely can be detected statistically. Effect concentrations associated with such small reductions have wide uncertainty bands, making them unreliable for criteria derivation. Adverse effects are generally modeled as a sigmoid function of increasing concentrations of the toxic substance (Figure 5).

Dose-Response Relationship
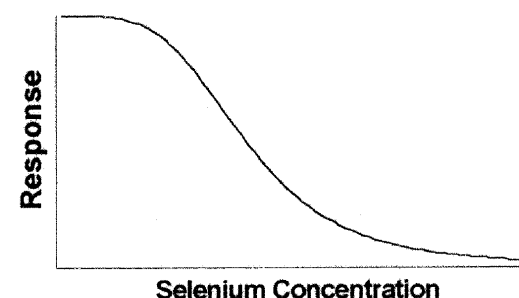

Selenium Concentration

Figure 5. Reductions in survival, growth or other responses of organisms are often modeled as a sigmoid function of increasing concentrations of selenium, or any other toxic substance.

A logistic regression was used to model negative effects of increasing concentrations of selenium on growth, survival, or percent of normal individuals (without deformities) of several aquatic species. The equations that described such functions were then used to estimate the concentration that promoted a 20 percent reduction in response observed at control levels (EC_{20}). These analyses were performed using the Toxic Effects Analysis Model software (version 0.02; R. Erickson, U.S. EPA Duluth).

Only data sets that met the following conditions were included in the analysis: (1) the experiment had a control treatment, which made it possible to define response levels at natural concentrations of selenium, (2) and at least four concentrations of selenium. (3) The highest tested concentration of selenium caused >50 percent reduction relative to the control treatment, and (4) at least one tested concentration of

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selenium caused <20 percent reduction relative to the control treatment to ensure that the EC_{20} was bracketed by tested concentrations of selenium. When the response was expressed as percentages (e.g., percent survival), transformed values (arcsin of the square root) were used to homogenize the variance.

When the data from an acceptable chronic test met the conditions for the logistic regression analysis, the EC_{20} was the preferred chronic value. When data did not meet the conditions, best scientific judgment was used to determine the chronic value. In this case the chronic value is usually the geometric mean of the NOAEC and LOAEC. But when no treatment concentration was an NOAEC, the chronic value is less than the lowest tested concentration. And when no treatment concentration was a LOAEC, the chronic value is greater than the highest tested concentration.

Logistic regression assumes that a logistic model describes the log dose-response curve. For a visual display of such model, a logistic curve with three parameters was fitted to each data set using nonlinear least-squares regression analysis (Draper and Smith 1981). The logistic model was

$$y = \frac{y_0}{1 + ax^b}$$

where x symbolizes the selenium concentration in the organism's tissues, y is the response of interest (survival, growth, or reproduction), and y_0, a and b are model parameters estimated by the regression analysis. The y_0 parameter represents the response of interest at background levels of selenium. The graphs also include the 95 percent confidence interval for projections of the logistic model. These tasks were performed in S-Plus version 6.0 (Insightful 2001).

Evaluation of Freshwater Chronic Data for Each Species

Acceptable freshwater chronic toxicity data are currently available for an aquatic invertebrate (*Brachionus calyciflorous*), six different fish species, and a mix of fish species from the family Centrarchidae; total of 17 different studies (Table 4). Detailed summaries of each study are included in Appendix H. Collectively, only these data were considered for the derivation of a final tissue residue criterion for selenium. Below is a brief synopsis of the experimental design, test duration, relevant test endpoints, and other critical information regarding the derivation of each specific chronic value. The chronic toxicity values for other chronic selenium toxicity values and endpoints are included in Appendix H.

Brachionus calyciflorus (freshwater rotifer)

This study reported by Dobbs et al. (1996) is one of two laboratory-based experiments (also see Bennett et al. 1986) that involved exposing algae to selenium (in this case as sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fish (fathead minnows). In this particular study, the rotifers and fish were exposed to the same concentrations of sodium selenate in the water as the algae, but received additional selenium from their diet (i.e., the algae fed to rotifers and the rotifers fed to fish). The overall exposure lasted for 25 days. Rotifers did not grow well at concentrations exceeding 108.1 μg Se/L in water, and the population survived only 6 days at selenium concentrations equal to or greater than 202.4 μg Se/L in the water (40 μg/g dw in the algae). Regression analysis of untransformed growth data (dry weight) determined 4 day post-test initiation resulted in a calculated EC_{20} of 42.36 μg Se/g dw tissue (Table 4).

Oncorhynchus tshawytscha (chinook salmon)

Hamilton et al. (1990) conducted a 90-day growth and survival study with swim-up larvae fed one of two different diets. The first diet consisted of Oregon moist pellets where over half of the salmon meal was replaced with meal from selenium-laden mosquitofish (*Gambusia affinis*) collected from the San Luis Drain, CA (SLD diet). The second diet was prepared by replacing half the salmon meal in the Oregon moist pellets with meal from low-selenium mosquitofish (i.e., the same relatively uncontaminated mosquitofish that were used in the control diet) and spiked with seleno-DL-methionine (SeMe diet). Analysis of the trace element composition in the two different diets indicated that while selenium was the most toxic element in the SLD diet, concentrations of boron, chromium, iron and strontium in the high-selenium mosquitofish replacement diet (SLD diet type) were slightly elevated compared to the replacement diet composed of uncontaminated control mosquitofish that were spiked with organic selenium (SeMe diet type). These trace elements were, however, only 1.2 (e.g., iron) to 2.0 times (e.g., chromium) higher in the SLD diet than the SeMe diet, which contained the following measured concentrations (dry weight basis) in the food: boron- 10 μg/g; chromium- 2.8 μg/g, iron- 776 μg/g, and strontium- 48.9 μg/g.

During the test, the survival of control chinook salmon larvae and larvae fed the lowest dietary selenium concentrations in either dietary exposure type (SLD and SeMe, respectively, consuming food at approximately 3 μg Se/g dw) exceeded ≥97 percent up to 60 days post-test initiation. Between 60 and 90 days of exposure, however, the control survival declined significantly. Therefore, only data collected up to 60 days post-test initiation was considered for analysis. Regression analysis of untransformed growth

data after 60 days of exposure resulted in a calculated EC_{20} of 15.74 µg Se/g dw tissue for fish fed the SLD diet type, and 10.47 µg Se/g dw tissue for fish fed the SeMe diet type (Table 4). Note: The mosquitofish from San Luis Drain were not tested for contaminants other than certain key elements suspected to be present in these fish. The San Luis Drain receives irrigation drainage from the greater San Joaquin Valley; and therefore, there is the possibility that the mosquitofish used in this study may have contained elevated levels of pesticides. The use of the SLD diet results assumes that selenium, and not these other possible contaminants, was the cause of any adverse chronic effects.

Oncorhynchus mykiss (rainbow trout)

Hilton and Hodson (1983) reared juvenile rainbow trout on either a high (25 percent) or low (1 percent) available carbohydrate diet supplemented with sodium selenite for 16 weeks. Body weights, feed:gain ratios, and total mortalities were followed throughout the exposure every 28 days. Tissues (livers and kidneys) were extracted for selenium analysis after 16 weeks. Fish fed the diets (low carbohydrate and high carbohydrate) with the highest selenium concentration (11.4 and 11.8 µg/g dw food, respectively) exhibited a 45 to 48 percent reduction in body weight (expressed as kg per 100 fish) compared to control fish by the end of the exposure, which the authors attributed to food avoidance. With only two dietary exposure concentrations and a control, these data were not amenable to regression analysis. The maximum acceptable toxicant concentration (MATC) for growth of juvenile rainbow trout relative to the final concentrations of selenium in liver tissue of trout reared on the high carbohydrate seleniferous dietary type is the geometric mean (GM) of 21.0 µg/g dw (NOAEC) and 71.7 µg/g dw (LOAEC), or 38.80 µg Se/g dw. Using the equation III to convert the selenium concentration in liver tissue to a concentration of selenium in the whole-body, the MATC becomes 9.659 µg/g dw (Table 4). The calculated MATC for the same group of experimental fish exposed to selenium in the low carbohydrate diet for an additional 4 weeks based on the occurrence of nephrocalcinosis in kidneys was estimated to be 10.42 µg Se/g dw tissue (see Hicks et al. 1984).

Hilton et al. (1980) employed a similar test design as Hilton and Hodson (1983) in a later experiment to examine the narrow window at which selenium changes from an essential nutrient to a toxicant affecting juvenile rainbow trout. The food consisted of a casein-torula yeast diet supplemented with selenium as sodium selenite. The experiment lasted for 20 weeks. During this time, the trout were fed to satiation 3 to 4 times per day, 6 days per week, with one feeding on the seventh day. Organs (liver and kidney) and carcasses were analyzed for selenium from fish sacrificed at 4 and 16 weeks. No gross histopathological or physiological effects were detected in the fish, although trout raised on the highest dietary level of

selenium (13.06 µg/g dw) had a significantly lower body weight (wet basis), a higher feed:gain ratio, and higher number of mortalities (10.7; expressed as number per 10,000 fish days). The MATC for growth and survival of juvenile rainbow trout relative to the final concentrations of selenium in whole-body tissue estimated from the selenium concentrations measured in the liver using the equation III is the GM of the NOAEC (9.710 µg/g dw tissue) and the LOAEC (22.31 µg/g dw tissue), or 14.72 µg/g dw tissue (Table 4).

Oncorhynchus clarki (cutthroat trout)

No significant effects of bioaccumulated selenium on mortalities and deformities in the eggs, larvae, and fry from wild-caught cutthroat trout from a reference and exposed site (Fording River, British Columbia, Canada) were observed by Kennedy et al. (2000). The observations were made on eggs reared in well water from spawning age females collected from the two locations (N = 17 and 20, respectively) and fertilized by one male collected at each site. The mean selenium content in muscle tissue from adult fish was 2.4 µg/g dw tissue for fish collected from the reference site, and 12.5 µg/g dw tissue for fish collected from the Fording River. Using Equation I to convert the selenium concentration in muscle tissue to a selenium concentration in the whole-body, the chronic value for this species was estimated to be >10.31 µg/g dw parental fish tissue (see Table 4).

Pimephales promelas (fathead minnows)

Chronic values for fathead minnows were derived from three laboratory-based studies and one mesocosm study (Table 4). Two of the laboratory studies (Bennett et al. 1986 and Dobbs et al. 1996) involved exposing algae to selenium (either as sodium selenite or sodium selenate) in water, and subsequently feeding the algae to rotifers which were in turn fed to fathead minnows. In the Bennett et al. (1986) study, larval fathead minnows were fed control (cultured in chambers without selenium containing algae) or selenium-contaminated rotifers (cultured in chambers with selenium containing algae previously exposed to sodium selenite in the water) in three separate experiments lasting 9 to 30 days. The different experiments were distinguished by: 1) the day selenium-laden rotifers were first fed, 2) the day selenium-laden rotifers were last fed, and 3) the age of larvae at experiment termination. The results from the three experiments reported by Bennett et al. (1986) were conflicting. Larval growth was significantly reduced at whole-body selenium concentrations ranging from 43.0 to 51.7 µg/g dw tissue in the first two experiments (see Appendix H for conditions), but growth was not significantly reduced in larvae that had accumulated 61.1 µg/g dw tissue in the third experiment (Table 4). The geometric mean of these three values, 51.40 µg/g dw, was considered the chronic value for selenium for this test.

A similar test system was used by Dobbs et al. (1996), in which larval fathead minnows were exposed to the same concentrations of sodium selenate in the water as their prey (rotifers), but also received additional selenium from the consumption of the selenium-contaminated rotifers, In this study, the fathead minnows did not grow well at concentrations exceeding 108.1 µg Se/L in water, and they survived only to 11 days at selenium concentrations equal to or greater than 393.0 µg/L in the water (75 µg Se/g dw in the diet, i.e., rotifers). The LOAEC for retarded growth (larval fish dry weight) in this study was <73 µg/g dw tissue (Table 4).

In contrast to the above laboratory-based food chain studies, Ogle and Knight (1989) examined the chronic effects of only elevated foodborne selenium on growth and reproduction of fathead minnows. Juvenile fathead minnows were fed a purified diet mix spiked with inorganic and organic selenium in the following percentages: 25 percent selenate, 50 percent selenite, and 25 percent seleno-L-methionine. The pre-spawning exposure lasted 105 days using progeny of adult fathead minnows originally obtained from the Columbia National Fishery Research Laboratory, and those obtained from a commercial fish supplier. After the 105 day exposure period, a single male and female pair from each of the respective treatment replicates were isolated and inspected for spawning activity for 30 days following the first spawning event of that pair. There was no effect from selenium on any of the reproductive parameters measured, including larval survival, at the dietary concentrations tested (5.2 to 29.5 µg/g dw food). Sub-samples of larvae from each brood were maintained for 14 days post-hatch and exhibited >87.4 percent survival. The pre-spawning adult fish fed a mean dietary level of 20.3 µg Se/g dw did exhibit a significant reduction in growth compared to controls (16 percent reduction), whereas no effect on growth occurred in the fish fed 15.2 µg/g dw. The whole-body chronic value, as determined by the GM of the NOAEC and the LOAEC measured at 98 days post-test initiation, was 5.961 µg/g dw tissue (Table 4).

The chronic value of 5.961 µg/g dw determined for growth after 98 days of exposure to pre-spawning fathead minnow adults (Ogle and Knight, 1989) was approximately an order of magnitude lower than the growth effects to fathead minnow observed in Bennett et al. (1986) and Dobbs et al (1996). The length of exposure in the Ogle and Knight test was more than twice as long as either Bennett et al. or Dobbs et al., suggesting a longer duration was needed in order to detect any growth effects from selenium. However, survival of larvae hatched from parents exposed to each of the five selenium treatments (including those in which growth was affected) was not affected.

Other studies (Bryson et al. 1984; Bryson et al. 1985a; Coyle et al. 1993; Hermanutz et al. 1996) have found larval deformities and larval survival to be the most sensitive endpoint to fish. This also appears true for fathead minnows. Schultz and Hermanutz (1990) examined the effects of selenium in fathead minnow larvae transferred from parental fish (females). The parental fathead minnows were originally exposed to selenite which was added to artificial streams in a mesocosm study. The selenite entered the food web which contributed to exposure from the diet. Spawning platforms were submerged into treated and control streams. The embryo samples that were collected from the streams were brought into the laboratory and reared in incubation cups which received stream water dosed with sodium selenite via a proportional diluter. Edema and lordosis were observed in approximately 25 percent of the larvae spawned and reared in natural water containing 10 µg Se/L. Selenium residues in the ovaries of females from the treated stream averaged 39.27 µg/g dw. Using equation II to convert the selenium concentration in the ovaries to a concentration of selenium in the whole-body, the chronic value for this species was estimated to be <18.99 µg/g dw (Table 4).

Since Ogle and Knight reported that food in the higher selenium concentrations remained uneaten and fish were observed to reject the food containing the higher selenium concentrations, the authors suggested that the decreased growth was caused by a reduced palatability of the seleniferous food items. This is a common observation also noted by Hilton and Hodson (1983) and Hilton et al. (1980) and apparent in Coughlan and Velte (1989). Given the no observed effect to larval survival and the apparent non-toxicological effect on growth in the Ogle and Knight study, the SMCV for fathead minnows does not include the 5.961 µg/g dw chronic value.

Lepomis macrochirus (bluegill sunfish)
Applicable chronic data for bluegill sunfish can be grouped according to field exposure versus laboratory exposure. In some field studies, chronic tolerance to selenium appears to be much higher than in laboratory studies (Bryson et al. 1985a; Lemly 1993b).

In the Bryson et al. (1984, 1985a) and Gillespie and Baumann (1986) studies, the progeny of females collected from a selenium contaminated reservoir, Hyco Reservoir, Person County, NC and artificially crossed did not survive to swim-up stage, irrespective of the origin of milt used for fertilization. Measured waterborne selenium concentrations prior to the experiments ranged from 35 to 80 µg/L. The whole-body tissue selenium concentration in the female parent associated with this high occurrence of mortality of hatched larvae was <43.32µg/g dw tissue, as reported by Bryson et al. (1985a), and <22.16

μg/g dw tissue, as reported by Gillespie and Baumann (1986) (Table 4). In the case of the latter, nearly all swim-up larvae from the Hyco Reservoir females were edematous, none of which survived to swim-up. These chronic effect tissue values are in line with the EC_{20} calculated for the occurrence of deformities among juvenile and adult fishes from the family Centrarchidae collected from Belews Lake, NC, i.e., 44.57 μg Se/g dw (see Lemly 1993b, Table 4).

In contrast, the chronic effects threshold for larval survival in a combination laboratory waterborne and dietary selenium exposure (Coyle et al. 1993), or even a long-term mesocosm exposure (Hermanutz et al. 1996), occurs at concentrations approximately 3 times lower than those recorded above (Table 4). In the Coyle et al. (1993) study, two-year old pond reared bluegill sunfish were exposed in the laboratory to a nominal 10 μg Se/L in water (measured concentrations in respective dietary treatments ranging from 8.4 to 11 μg/L) and fed (twice daily *ad libitum*) Oregon moist pellets containing increasing concentrations of seleno-L-methionine. The fish were grown under these test conditions for 140 days. Spawning frequency, fecundity, and percentage hatch were monitored after 60 days when spawning began to occur. There was no effect of the combination of the highest dietary selenium concentration (33.3 μg Se/g dw) in conjunction with waterborne selenium concentrations averaging 11 μg/L on adult growth, condition factor, gonadal somatic index, or the various reproductive endpoints (Appendix H). The survival of newly hatched larvae, however, was markedly reduced; only about 7 percent survived to 5 days post-hatch. Regression analysis on arcsin square root transformed fry survival data 5 days post-hatch resulted in a calculated EC_{20} of 8.95 μg Se/g dw tissue (Table 4).

Hermanutz et al. (1996), as corrected by Tao et al. (1999), exposed bluegill sunfish to sodium selenite spiked into artificial streams (nominal test concentrations: 0, 2.5, 10, and 30 μg Se/L) which entered the food web, thus providing a simulated field-type exposure (waterborne and dietary selenium exposure). A series of three studies were conducted over a 3 year period lasting anywhere from 8 to 11 months. Spawning activity was monitored in the stream, and embryo and larval observations were made *in situ* and from fertilized eggs taken from the streams and incubated in egg cups in the laboratory. None of the adult bluegill exposed to the highest concentration of selenium in the water (mean measured concentration equal to 29.4 μg/L) survived. Incidence of edema, hemorrhage, and lordosis in the larvae incubated in egg cups and spawned from fish exposed to 10 μg Se/L were 100, 45 and 15 percent, respectively (see Hermanutz 1996 in Appendix H). Such health problems were not observed in larvae from fish that were not exposed to elevated concentrations of selenium (control treatment). Rates of edema, hemorrhage, and lordosis occurrence in larvae (egg cup data) from fish exposed to 2.5 μg Se/L

The importance of diet in the bioaccumulation of selenium was demonstrated in one additional experiment. Study III consisted of the addition of new adult bluegill to the same streams that received the 2.5, 10 and 30 μg/L sodium selenite during previous studies, but with all dosing of selenite halted. The adult bluegills exposed only to dietary selenium present in the food web accumulated selenium to levels very near to the levels accumulated during Study II in which aqueous selenium was also present demonstrating the importance of diet on selenium accumulation. There were no effects (no effect on larval survival, 0 percent deformities, 0 percent hemorrhaging), on the bluegill progeny in Study III even from fish that accumulated 11.7 and 14.5 μg/g dw in the recovering 10 μg/L streams, and 17.3 μg/g dw in the recovering 30 μg/L stream. The lack of any effect on the Study III larvae suggests bluegill are more sensitive to a combined aqueous and dietary selenium exposure than they are to dietary only selenium.

Data from Lemly (1993a) indicate that over-wintering fish may be more susceptible to the effects of waterborne and dietary selenium due to increased sensitivity at low temperature. The authors exposed juvenile bluegill sunfish in the laboratory to waterborne (1:1 selenite:selenate; nominal 5 μg Se/L) and foodborne (seleno-L-methionine in TetraMin; nominal 5 μg Se/g dw food) selenium for 180 days. Tests with a control and treated fish were run at $4^{\circ}C$ and $20^{\circ}C$ with biological and selenium measurements made every 60 days. Survival, whole-body lipid content, and oxygen consumption were unaffected compared to control fish exposed at $20^{\circ}C$ (whole-body selenium concentrations equal to 6 μg/g dw), whereas fish exposed to the combination low-level waterborne and dietary selenium at $4^{\circ}C$ exhibited significantly elevated mortality (33.8 percent) relative to controls (2.7 percent), and exhibited significantly greater oxygen consumption and reduced lipid content, which are all indicative of an additional stress load. The chronic value for juvenile bluegill sunfish exposed to waterborne and dietary selenium at $4^{\circ}C$ was <7.9 μg/g dw tissue.

Five of the studies discussed above evaluated the effects of selenium on fish larvae to which exposure was through the parents. Three of these studies collected adult fish from Hyco Reservoir to which the bluegill population had been exposed to elevated selenium concentrations for multiple generations (Bryson et al. 1984; Bryson et al. 1985a; Gillespie and Baumann 1986), whereas the other two studies exposed bluegill parents obtained from an uncontaminated source (Coyle et al. 1993; Hermanutz et al. 1996). The average of the chronic values reported for the Hyco studies were four times the values in the latter two studies. This difference may simply be the inability of the field tests to evaluate a lower effect concentration than that which occurs at the site. However, Bryson et al. (1985a) found no effects to larval survival from Hyco Reservoir females collected in an "unaffected area" containing 19.18 μg/g dw

suggesting the possibility of tolerance through physiological or genetic adaptation of the previous exposed bluegill population at Hyco Reservoir.

Acquisition of tolerance to selenium has also been implied in the literature for other fish species. For example, Kennedy et al. (2000) suggested that the cutthroat trout collected from a stream containing 13.3 to 14.5 µg Se/L in the water column were tolerant at the cellular level explaining their ability to develop normally in the early life stages. Kennedy et al. reported the overall frequency of larval deformities in the exposed population was less than 1 percent, and in one fish containing eggs with 81.3 µg/g dw, there were 0.04 percent pre-ponding deformities and 3.3 percent larval mortalities. Other than the Kennedy et al. study, tolerance to selenium at the apparent most sensitive endpoint to fish, embryo-larval development, has not been reported in the literature and its reality is uncertain at this time. However, given the need to protect sensitive populations of species, the chronic values for the studies in which eggs and larvae were obtained from bluegill adults that were exposed to elevated selenium for multiple generations (i.e., Bryson et al. 1984; Bryson et al. 1985a; Gillespie and Baumann, 1986) were not included in the SMCV calculation.

Morone saxitilis (Striped bass)

The only remaining applicable chronic value for selenium was determined from a laboratory dietary exposure conducted using yearling striped bass (Coughlan and Velte 1989). During the experiment, the bass were fed contaminated red shiners (38.6 µg Se/g dw tissue) from Belews Lake, NC (treated fish) or golden shiners with low levels of selenium (1.3 µg/g dw tissue) purchased from a commercial supplier (control fish). The test was conducted in soft well water and lasted up to 80 days. During the experiment, all fish were fed to satiation 3 times per day. Control fish grew well and behaved normally. Treated fish behaved lethargically, grew poorly due to a significant reduction in appetite, and showed histological damage, all eventually leading to the death of the animal. The final selenium concentration in muscle of treated striped bass averaged from 17.50 to 20.00 µg/g dw tissue (assuming 80 percent moisture content), which was 3.2 to 3.6 times higher than the final selenium concentrations in control striped bass, which averaged 5.500 µg/g dw tissue. Using equation I to convert the selenium concentration in muscle tissue to a selenium concentration in the whole-body, the chronic value for this species was determined to be <17.50 µg/g dw (Table 4).

Formulation of the Final Chronic Value (FCV) for Selenium

The lowest GMCV in Table 4 is for bluegill, 9.5 µg/g dw whole body, which is the geometric mean of chronic values from the laboratory study of Coyle et al. (1993), the laboratory study of Lemly (1993a), and the macrocosm exposure study of Hermanutz et al. (1996). The "less than" values tabulated for Bryson et al. (1984) and Gillespie and Baumann (1986) for Hyco Reservoir bluegill did not contribute to this mean because they only indicate a chronic value in a range that includes 9.5 µg/g dw.

The Table 4 results for Bryson et al. (1985a) and Lemly (1993b) were also not used in calculating the bluegill GMCV. Bryson et al. (1985a) indicated a chronic value for Hyco Reservoir bluegill somewhere between 19.18 and 43.43 µg/g dw. Lemly (1993b), appearing in Table 4 under the category Centrarchidae, the family that includes bluegill, yielded a Belews Lake chronic EC20 of 44.57 µg/g dw, again substantially above the GMCV of 9.5 µg/g dw. It is not known whether historical exposure to elevated selenium concentrations, such as occurred at Belews Lake and Hyco Reservoir, will dependably lead to this magnitude of increase in the chronic tolerance of resident fish.

The Lemly (1993a) laboratory results, indicating a chronic value <7.9 µg/g dw, are not completely comparable to the other results used to calculate the bluegill GMCV. Lemly (1993a) involved an additional natural stress, exposure to a winter low temperature of 4°C. This appeared to reduce the tissue concentration associated with reduced survival. Because this stress occurs annually to one degree or another in nearly all the country, the FCV was lowered to 7.9 µg/g dw. Although the literature contains little information on the temperature-dependence of selenium toxicity, Lemly's study (further summarized in Appendix H) was judged to be sufficiently definitive to merit lowering the FCV.

The Guidelines indicate that the chronic criterion (in this case the FCV) is intended to be a good estimate of the threshold for unacceptable effect. The Guidelines point out that the threshold for unacceptable effect does not equate with a threshold for any adverse effect. Some adverse effects, possibly even a small reduction in survival, growth, or reproduction, may occur at this threshold. If bluegill is as sensitive as indicated by the Lemly (1993a) results, a minor reduction in survival (compared to populations accumulating lesser concentrations of selenium or exposed to less severe winter temperatures) would occur at the FCV. Nevertheless, other studies, those of Lemly (1993b) and Bryson et al. (1985a), suggest that historically exposed populations would not be as sensitive as the organisms studied by Lemly (1993a).

The FCV may not necessarily protect fish in artificial environments where they are exposed only via water and not via diet. If the organisms are provided with an uncontaminated diet, then exceedingly high water concentrations, possibly above the acute criterion, are needed to elicit effects, but such effects may occur at tissue concentrations below the FCV (Cleveland et al. 1993; Gissel-Nielsen and Gissel-Nielsen 1978). This is not a practical limitation, however, since water-only exposure of selenium is not representative of the actual exposure of selenium to aquatic organisms in the environment.

Although this aquatic life criterion was not developed with the intent of protecting terrestrial wildlife, the FCV is expected to be protective of birds dependent on an aquatic food chain. Adverse effects to waterfowl, shorebirds and piscivorus birds have been associated with elevated selenium concentrations at several western locations, notably at Kesterson Reservoir in the San Joaquin Valley, California (Burton et al. 1987b; Horne 1991; Ohlendorf 1986; Ohlendorf et al. 1986a,b; Saiki 1986a,b). An effect level was determined in the laboratory by Heinz et al. (1987) through feeding adult mallards and their ducklings food that contained selenite or selenomethionine. The number of 21-day old ducklings per hen was 9.7 for the controls and 2.0 for the animals that received food containing 10 µg/g selenomethionine. The treatments receiving 10 and 25 µg/g selenite produced 8.1 and 0.2 ducklings per hen, respectively. Food containing 10 µg/g selenomethionine resulted in nearly ten times as much selenium in eggs as did food containing 10 µg/g selenite. Selenomethionine resulted in more selenium in egg white than yolk, but the opposite was true for selenite. Adult mallards fed diets containing 10 µg/g seleno-DL-methionine for 76 days (Heinz and Hoffman 1998) displayed reduced hatching success, reduced survival of ducklings and produced a higher percentage of deformities when compared to the control group. Adults exposed under control conditions produced an average of 7.6 young per female, and 6.1 percent of the embryos had deformities. Females fed 10 µg/g selenomethionine produced an average of 2.8 young and 36.2 percent of the embryos had deformities.

A way to estimate risk to birds is to compare the FCV to effect levels derived for selenium in the diet of piscivorus birds. Opresko et al. (1995) derived chronic No Observed Adverse Effect Levels (NOAEL) and Lowest Observed Adverse Effect Levels (LOAEL) for three piscivorus birds: belted kingfisher, great blue heron and osprey, using the mallard data generated by Heinz et al. (1987). From the NOAELs and LOAELs, they calculated the dietary concentration in food of the contaminant that would result in a dose equivalent to the NOAEL and LOAEL (assuming no exposure through other environmental media). The chronic values for these birds, including the GM of the two dietary levels, are given in the following table:

Dietary Levels[a] for Selenite			
Species	dietary level that would result in a dose equivalent to the NOAEL, µg/g dw	dietary level that would result in a dose equivalent to the LOAEL, µg/g dw	dietary level that would result in a dose equivalent to the MATC, µg/g dw
belted kingfisher	9.5	18.5	13.26
great blue heron	10.5	21.5	15.02
osprey	11	22	15.56

Dietary Levels[a] for Selenomethionine			
Species	dietary level that would result in a dose equivalent to the NOAEL, µg/g dw	dietary level that would result in a dose equivalent to the LOAEL, µg/g dw	dietary level that would result in a dose equivalent to the MATC, µg/g dw
belted kingfisher	7.5	15	10.61
great blue heron	8.5	17	12.02
osprey	8.5	17.5	12.20

a   Converted from wet weight to dry weight using a moisture content of 0.80 (U.S. EPA 1985b).

Comparing the FCV with the dietary levels that would result in a dose equivalent to the MATC indicates piscivorus birds would be protected from unacceptable effects if their diet (fish) is maintained or kept below the FCV. This assessment assumes that there is minimal exposure of selenium from other sources. Opresko et al. (1995) estimate the concentration of selenium in water needed to produce effects at the NOAEL and LOAEL for these birds ranges from 6,800 to 8,700 µg/L, which is approximately 1000 times the concentration of waters in which fish would be approaching the FCV level. Exposure of selenium to these birds through the intake of water at 1,000 times lower than the effect level would therefore be a minimal exposure.

FCV Relative to Natural Background Levels of Selenium in Fish

As an essential element, selenium naturally occurs in all living things. Since selenium is found in all fish, two questions arise. 1) How close is the FCV of 7.9 µg/g dw to natural background levels in fish, and 2) how frequently do natural selenium tissue concentrations exceed the FCV. The latter situation would pose problems in the implementation of the FCV as an ambient water quality criterion.

As part of the National Contaminant Biomonitoring Program, the U.S. Fish and Wildlife Service collected fish from 112 sites distributed evenly across the U.S. during 1979 through 1981 and measured several contaminants including selenium (Lowe et al. 1985). Selenium, measured in 591 fish representing 60 different species, ranged from 0.3 to 10.5 µg/g dw and had an overall average and standard deviation of 1.9 ± 1.4 µg/g dw.

A separate data set of selenium measured in macroinvertebrates and fish collected from 48 reference sites in USGS's National Water Quality Assessment (NAWQA) program. NAWQA is intended to measure water quality in a sampling of smaller watersheds having known land use. The categories of such land use span a wide range, and include residential, industrial, agricultural, and mixed, among others. The 48 sites evaluated for this comparison excluded watersheds with land use listed as anything other than "reference". Among these reference sites, whole body fish tissue concentrations ranged from 0.7 to 9.83 µg/g dw and had an overall average and standard deviation of 2.99 ± 1.96 µg/g dw. The distribution of both these data sets indicates that the FCV would not be in the range of natural background concentration for selenium in over 98 percent of fish collected across the United States (Figure 6; Appendix I). The FCV is therefore sufficiently greater than natural selenium levels that unavoidable exceedances of the criterion are unlikely.
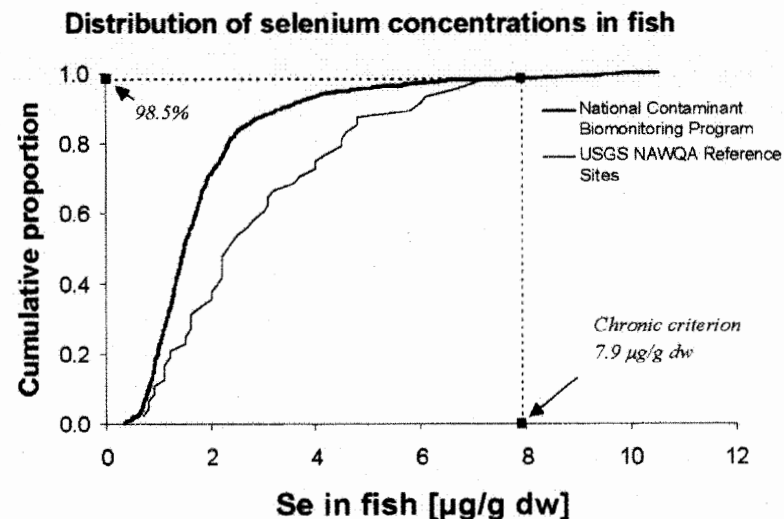

Figure 6.   Cumulative distribution of selenium (whole-body, µg/g dw) in 591 fish samples from 112 sites across the United States. From Lowe et al. 1985.

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Table 4.   Freshwater Chronic Values from Acceptable Tests

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, μg/g dw*	SMCV μg/g dw	GMCV μg/g dw
Brachionus calyciflorus rotifer	Dobbs et al. 1996	dietary and waterborne (lab)	algae exposed to SeVI in water, algae then fed to rotifers	EC_{20} for rotifer dry weight after 4 d	42.36	42.36	42.36
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab)	Se-laden mosquitofish from San Luis Drain, CA	EC_{20} for juvenile growth	15.74 (juvenile tissue)		
Oncorhynchus tshawytscha chinook salmon	Hamilton et al. 1990	dietary (lab)	Mosquitofish spiked with seleno-DL-methionine	EC_{20} for juvenile growth	10.47 (juvenile tissue)	12.84	>11.64
Oncorhynchus mykiss rainbow trout	Hilton and Hodson 1983; Hicks et al. 1984	dietary (lab)	sodium selenite in food preparation	MATC for juvenile growth; nephrocalcinosis	9.659[b] (juvenile tissue)		
Oncorhynchus mykiss rainbow trout	Hilton et al. 1980	dietary (lab)	sodium selenite in food preparation	MATC for juvenile survival and growth	14.72[c] (juvenile tissue)	11.92	
Oncorhynchus clarki cutthroat trout	Kennedy et al. 2000	dietary and waterborne (field - Fording River, BC)	not determined	Chronic value for embryo/larval deformities and mortality	>10.31[e] (parent tissue)	>10.31	
Pimephales promelas fathead minnow	Bennett et al. 1986	dietary (lab)	algae exposed to selenite then fed to rotifers which were fed to fish	Chronic value for larval growth	51.40 (larval tissue)		
Pimephales promelas fathead minnow	Ogle and Knight 1989	dietary (lab)	mix of 25, 50, and 25 percent selenate, selenite, and seleno-L-methionine in food preparation	MATC for pre-spawning adult growth	5.951[d] (pre-spawning adult tissue)	41.46	41.46
Pimephales promelas fathead minnow	Dobbs et al. 1996	dietary and waterborne (lab)	algae exposed to selenate in water then fed to rotifers which were fed to fish	LOAEC for larval fish dry weight after 8 d	<73 (larval tissue)		
Pimephales promelas fathead minnow	Schultz and Hermanutz 1990	dietary and waterborne (mesocosm - Monticello)	selenite added to artificial streams which entered food web and provided dietary exposure	Chronic value for larval edema and lordosis	<18.99 (parent tissue)		
Lepomis macrochirus bluegill	Bryson et al. 1984	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	Chronic value for larval mortality	<61.07[c,d] (parent tissue)		
Lepomis macrochirus bluegill	Bryson et al. 1985a	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	Chronic value for swim-up larvae	<43.32[c,d] >19.18[c,d] (parent tissue)		
Lepomis macrochirus bluegill	Gillespie and Baumann 1986	dietary and waterborne (field - Hyco Reservoir, NC)	not determined	Chronic value for larval survival	<28.20[d] (larval tissue), or <22.16[d,e] (parent tissue)	9.500	9.500
Lepomis macrochirus bluegill	Coyle et al. 1993	dietary and waterborne (lab)	diet: seleno-L-methionine water: 6:1 selenate:selenite	EC_{20} for larval survival	8.954 (parent tissue - females only)		
Lepomis macrochirus bluegill	Lemly 1993a	dietary and waterborne (lab)	diet: seleno-L-methionine water: 1:1 selenate:selenite	Chronic value for juvenile mortality	<7.9 (juvenile tissue)		

The available data for selenium, evaluated using the procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" (Stephan et al. 1985) indicate that, except possibly where an unusually sensitive species is important at a site, freshwater aquatic life should be protected if the concentration of selenium in whole-body fish tissue does not exceed 7.9 µg/g dry weight, and if the short-term average concentration of selenium dissolved in the water seldom exceeds 185 µg/L.

The available data for selenium, evaluated as above, indicate that saltwater aquatic life should likewise be protected if the short-term average concentration of dissolved selenium seldom exceeds 127 µg/L. If selenium is as chronically toxic to saltwater fishes as it is to freshwater fishes, the status of the fish community should be monitored if selenium exceeds 7.9 µg/g dw in the whole-body tissue of salt water fishes.

Species	Reference	Exposure route	Selenium form	Toxicological endpoint	Chronic value, µg/g dw*	SMCV µg/g dw	GMCV µg/g dw
Lepomis macrochirus bluegill	Hermanutz et al 1996	dietary and waterborne (mesocosm - Monticello)	selenite added to artificial streams which entered food web and provided dietary exposure	LOAEC for larval survival, edema, lordosis and hemorrhaging	12.12 (parent tissue)		
Centrarchidae (9 species)	Lemly 1993b	dietary and waterborne (field - Belews Lake, NC)	not determined	EC₂₀ for deformities among juveniles and adults	44.57 (juvenile and adult tissue)	NA	NA
Morone saxitilis striped bass	Coughlan and Velte 1989	dietary (lab)	Se-laden shiners from Belews Lake, NC	Chronic value for survival of yearling bass	<17.50ᵉ (juvenile tissue)	<17.50	<17.50

ᵃ All chronic values reported in this table are based on the measured or estimated (see footnotes below) concentration of selenium in whole body tissue.
ᵇ Estimated using the equation III.
ᶜ Estimated using the equation I.
ᵈ Chronic value not used in SMCV calculation (see text).
ᵉ Estimated using the equation II.

Implementation

As discussed in the Water Quality Standards Regulation (U.S. EPA 1983b), a water quality criterion for aquatic life has regulatory force only after it as been adopted in a state or tribal water quality standard. Such a standard specifies a criterion for a pollutant that is consistent with a particular designated use. With the concurrence of the U.S. EPA, states and tribes designate one or more uses for each body of water or segment thereof and adopt criteria that are consistent with the uses (U.S. EPA 1983c, 1987b). In each standard, a state or tribe may adopt the national criterion (if one exists), or an adequately justified state-specific or site-specific criterion.

State-specific or site-specific criteria may include not only criterion concentrations (U.S. EPA 1983c), but also state-specific or site-specific, and possibly pollutant-specific, durations of averaging periods and frequencies of allowed excursions (U.S. EPA 1985c). Because the chronic criterion is tissue-based for selenium, the averaging period only applies to the acute criterion, which is defined as a short-term average, based on the nature of the toxicity tests used for its derivation, and the speed at which effects may occur in such tests. Implementation guidance on using criteria to derive water quality-based effluent limits is available in U.S. EPA (1985c and 1987b).

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Report on the Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation

September 1998

REPORT ON THE
PEER CONSULTATION WORKSHOP ON
SELENIUM AQUATIC TOXICITY AND BIOACCUMULATION

September 1998

Office of Water
U.S. Environmental Protection Agency
Washington, DC

NOTE

This report was prepared by Eastern Research Group, Inc., a contractor to the U.S. Environmental Protection Agency (EPA), as a general record of discussion during the peer consultation workshop. As requested by EPA, this report captures the main points of scheduled presentations and discussions, and a summary of comments offered by observers attending the workshop; the report is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. This report will be used by EPA as an early scientific assessment of technical issues associated with selenium aquatic toxicology and bioaccumulation and will serve as a technical resource during EPA's review of freshwater selenium aquatic life criteria. The information in this document does not necessarily reflect the policy of the U.S. Environmental Protection Agency and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ACKNOWLEDGMENTS

This document summarizes the proceedings and presentations made at a 2-day workshop sponsored by the U.S. Environmental Protection Agency (EPA) to discuss selenium aquatic toxicology and bioaccumulation. The meeting was chaired by Anne Fairbrother of ecological planning and toxicity, inc., who wrote the overall meeting summary section and led one of the discussion sessions. Other discussion leaders included William Adams (Kennecott Utah Copper Corporation), Steven Hamilton (U.S. Geological Survey) and William Van Derveer (Colorado Springs Utilities). Technical presentations were made by A. Dennis Lemly (Virginia Tech University) and George Bowie (Tetra Tech, Inc.). Keith Sappington of EPA's Office of Water served as the Work Assignment Manager for this task. Kate Schalk, Rebekah Lacey, Lauren Lariviere, and Beth O'Connor of Eastern Research Group provided support services to plan and coordinate the workshop and prepare a summary report for task 98-09 under EPA Contract No. 68-D5-0028.

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Copies of this document may be obtained by contacting the U.S. EPA, National Center for Environmental Publications and Information (NCEPI), 11029 Kenwood Road, Cincinnati, Ohio, 45242, phone (513) 489-8190. In addition, the document will soon be published on the world wide web at http://www.epa.gov/ost/selenium.

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PREFACE

Under section 304(a) of the Clean Water Act, the U.S. Environmental Protection Agency (EPA) publishes ambient water quality criteria which serve as guidance to States and Tribes for setting enforceable water quality standards. Water quality standards form the basis for establishing pollutant discharge limits under the National Pollutant Discharge Elimination System (NPDES) and for setting Total Maximum Daily Loads (TMDLs). Given the importance of 304(a) criteria to the regulation of pollutant discharges to the Nation's waters, these criteria must be reviewed and revised periodically to reflect the latest scientific information.

Selenium is one chemical for which 304(a) aquatic life criteria have been derived, but which is currently undergoing review by EPA. Selenium exhibits a number of chemical and toxicological properties that complicate the derivation of numeric aquatic life criteria. Among these are: (1) its existence in at least four different oxidation states in the aquatic environment, (2) its propensity to bioaccumulate in aquatic food webs, and (3) its ability to convert between different chemical forms.

On May 27 and 28, 1998, EPA sponsored a workshop entitled: *Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation.* The goal of this peer consultation was to obtain early assessment of the state of the science on various technical issues associated with deriving aquatic life criteria for selenium. This document presents the proceedings from this workshop and is considered by EPA to be a valuable technical resource for future refinement of EPA's aquatic life criteria for selenium.

I. INTRODUCTION

Background

Selenium, a metalloid that is released to water from both natural and anthropogenic sources, can be highly toxic to aquatic life at relatively low concentrations. Selenium is also an essential trace nutrient for many aquatic and terrestrial species. Derivation of aquatic life criteria for selenium is complicated by its complex biogeochemistry in the aquatic environment. Specifically, selenium can exist in several different oxidation states in water, each with varying toxicities, and can undergo biotransformations between inorganic and organic forms. The biotransformation of selenium can significantly alter its bioavailability and toxicity to aquatic organisms. Selenium also has been shown to bioaccumulate in aquatic food webs, which makes dietary exposures to selenium a significant exposure pathway for aquatic organisms.

The most recent aquatic criteria for selenium were derived by the U.S. Environmental Protection Agency (EPA) in 1987. At the time of their publication, these criteria could not be conveniently adjusted to account for the combined toxicities of different selenium forms. Since then, a substantial body of literature has accumulated on the aquatic toxicity of different selenium forms (in combination and in isolation). In response to this and other new information, EPA has initiated an effort to evaluate and revise acute and chronic aquatic life criteria and site-specific criteria guidelines for selenium.

As part of this effort, EPA sponsored a Peer Consultation Workshop on Selenium Aquatic Toxicity and Bioaccumulation on May 27-28, 1998. This workshop brought together nine experts on the aquatic chemistry and biology of selenium to discuss technical issues underlying the freshwater aquatic life chronic criterion. The discussion among the experts was guided by questions posed in a technical charge written by EPA. While focusing on issues related to the chronic criterion, the charge also touched on technical questions pertinent to acute criteria, wildlife criteria, and site-specific criteria guidelines. The output from this meeting (recommendations in response to the technical charge) will be considered by an EPA-established work group that will be responsible for revising freshwater selenium criteria and for developing guidance for site-specific criteria.

Before the workshop, the experts submitted individual responses to the questions in the technical charge. At the workshop, the experts heard presentations by two leading selenium researchers; they then collectively discussed the questions in the technical charge and related issues. This report presents the results of this peer consultation. Section II of this report presents the chair's summary of the overarching themes and recommendations that emerged from the workshop. Section III summarizes the discussions and specific conclusions concerning each question in the technical charge. Section IV summarizes comments presented by observers at the meeting. Section V lists the references cited in the report.

Workshop materials, including the agenda and lists of experts, presenters, and observers, are provided in Appendix A. Appendix B includes the technical charge to the experts and background materials. Appendix C presents the experts' premeeting comments. Additional references provided by experts, presentation materials, and observer presentations are included in Appendices D, E, and F respectively.

Summary of Opening Remarks

Dr. Jeanette Wiltse, director of the Health and Ecological Criteria Division of EPA's Office of Water, opened the meeting and welcomed participants. She said that the peer consultation process allows EPA to

benefit from the knowledge and experience of experts in the field, obtaining better understanding of the problem and new perspectives. She thanked the experts for their time and effort.

Dr. Wiltse commented that metals present a technically complex problem when developing water criteria. One key issue is the balance between sufficiency and toxicity: Many metals (including selenium) are required by organisms in small amounts, but are toxic in larger amounts. She predicted that the experts would find the selenium discussion challenging and thanked them again for participating in the consultation.

Keith Sappington, also of the Health and Ecological Criteria Division, then presented an overview and background of the revision of EPA's freshwater aquatic life criteria for selenium. He said that the purpose of the consultation was to provide an early assessment of the science on a number of the technical issues associated with the criteria, and that EPA would use this information as a basis for moving forward through the criteria revision process. He explained that the impetus for EPA's review of the selenium criteria included:

- New data and concern over the level of protection (too high or too low?).

- Ecological importance (as selenium is both an essential trace nutrient and a toxicant).

- The need to address the toxicity and bioavailability of different selenium forms.

- The need for site-specific criteria modification procedures (taking into account bioaccumulation and food-web exposure).

He added that some fundamental issues EPA is facing in the development of the new criteria include determining in which environmental compartment to express the criteria, establishing the duration of the averaging period, and identifying the key factors affecting the toxicity and bioaccumulation of selenium.

Mr. Sappington emphasized that the focus of the peer consultation would be on technical issues underlying the freshwater aquatic life chronic criterion. He reminded the experts that discussion of risk management or policy decisions would not be appropriate to this forum. He discussed the key steps that EPA would undertake in its criteria review process and concluded by presenting a rough timeline for the development of the revised criteria. (See Appendices B and E for more detail.)

Dr. Anne Fairbrother, the workshop chair, then discussed the workshop structure and objectives, reminding experts again to focus only on reviewing the state of the science; she added that waterbirds would not be considered in the discussion. (See Appendix E for presentation materials.)

Opening Presentations

Belews Lake: Lessons Learned

Dr. A. Dennis Lemly of the Department of Fisheries and Wildlife at Virginia Tech University gave a presentation entitled "Belews Lake: Lessons Learned." (See Appendix E for presentation materials.) Belews Lake is a reservoir in the northwestern Piedmont area of North Carolina. The reservoir is hydrologically divided by a highway crossing into a main lake and the "158-Arm." The main lake received selenium input from disposal of waste ash from a coal-fired power plant. Inputs occurred over a 10-year

period, stopping in 1985. The combination of a period of ongoing inputs and a period of declining selenium concentrations has allowed researchers to obtain a great deal of information on tissue residue levels and effects. Dr. Lemly's summary of the key information gained from research at Belews Lake is as follows:

Main Lake Studies:
A concentration of ~10 μg/L dissolved selenium (about 80-90% selenite as it entered the lake) can bioaccumulate in aquatic food chains and cause massive reproductive failure in warm-water fish. Centrarchids (e.g., largemouth bass, bluegill, crappie, sunfish) are among the most sensitive to elevated selenium; forage species such as red shiners, fathead minnows, and mosquitofish are relatively tolerant (Cumbie and Van Horn, 1978; Lemly, 1985).

Once ecosystem equilibration to ~10 μg/L has occurred in this type of a reservoir setting, natural removal/cleansing processes operate very slowly. Elevated residues and toxic (teratogenic) effects in fish were evident 10 years after selenium inputs stopped and waterborne concentrations dropped below 1 μg/L (Lemly, 1997); consumption advisories are still in effect because of public health concerns. Complete recovery can be on the order of decades.

Dietary selenium was the most important source leading to effects in fish. Across years, the sediment/detrital route of exposure delivered the most consistent dose to fish (i.e., residues in benthos were consistently high). However, within a given year, residues in the waterborne/planktonic route of exposure were occasionally as high as in the benthic pathway (70-90 μg/g dry weight, especially in summer). Thus, each route of exposure delivered a toxic dose to fish. Planktivores, omnivores, insectivores, and piscivores were all similarly affected.

158-Arm Studies:
Concentrations of 0.2-4 μg/L dissolved selenium in the 158-Arm bioaccumulated to levels that caused teratogenic deformities and chronic selenosis (pathological lesions) in sensitive fish species (e.g., bluegill and green sunfish) (Sorensen et al., 1984; Lemly, 1993a, 1997).

Concentrations of 0.2-4 μg/L dissolved selenium bioaccumulated to >25 μg/g dry weight in aquatic food-chain organisms. This concentration is over five times the chronic dietary toxicity threshold for freshwater fish and aquatic birds, as determined in laboratory studies (i.e., 3-5 μg/g; Lemly 1993b).

Selenium concentrations in fish (especially bluegill) reached levels equal to or greater than those that caused reproductive failure in artificial crosses of bluegill from a sister lake (Hyco Reservoir; 38-54 μg/g dry weight whole body concentrations in fish; Cumbie and Van Horn, 1978; Holland, 1979; Gillespie and Baumann, 1986), and reproductive failure in laboratory feeding experiments with bluegill (13 and 33 μg/g dry weight in fish diets; Woock et al., 1987; Coyle et al., 1993).

Related Laboratory Studies:
Exposure to waterborne (only) selenium (selenite) at concentrations of 10 μg/L does not affect survival of juvenile bluegill. Although some bioconcentration occurs, residues in tissues do not reach the toxic threshold (Lemly, 1982).

Conditions mimicking those in the Belews 158-Arm (4-5 μg/L dissolved selenium; 5 μg/g dry weight dietary selenium) can induce physiological and metabolic stress in young centrarchids, resulting in

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significant mortality during cold weather due to Winter Stress Syndrome (Lemly, 1993c, 1996). Thus, time of year may be an important factor in the toxicity process when concentrations are near the current EPA criterion for chronic exposure (5 µg/L).

Conclusions:

Because of the extensive and rapid collapse of fish populations, the main body of Belews Lake has received most of the research focus and notoriety. However, the 158-Arm provides valuable information on selenium bioaccumulation and effects when waterborne concentrations are below the EPA national criterion for chronic exposure (5 µg/L).

Historic and current reference to the 158-Arm as "unaffected" (e.g., EPA 1998 Draft Field Study Summary) are incorrect. Multiple lines of evidence from this field site, (diagnostic residues, tissue pathology, teratogenic deformities) as well as associated laboratory studies (simultaneous water/diet exposures), indicate that selenium can become toxic to fish when waterborne concentrations are 4 µg/L or less. The affected taxa include widely distributed, economically and recreationally important species such as largemouth bass and bluegill. In this type of field setting, the threshold for detrimental impacts is well below 5 µg/L.

The most sensitive biological endpoint for detecting toxicity in fish (that has demonstrated impacts at a population and community level) is reproductive failure (i.e., teratogenic deformities and associated embryomortality that occur shortly after hatching). Winter Stress Syndrome may be a more sensitive indicator but it has not been confirmed in field studies.

From a toxicity perspective, the point of effect is the fish's reproductive tissue (i.e., eggs). The toxic threshold for selenium in eggs (10 µg/g dry weight) is consistent regardless of the source or chemical form of selenium in an aquatic system. Pairing water and egg concentrations gives a direct source-fate, cause-effect linkage that integrates all aspects of the selenium cycle. The existing national field database suggests that a single water-tissue method for setting criteria can be applied equally to both selenate and selenite dominated systems.

The practice of allowing exceedances in meeting water quality criteria is not supported by field evidence of effects. For example, current EPA guidelines allow up to 20 µg/L as an ambient (lake-wide) concentration once every 3 years. The concentration of waterborne selenium in Belews Lake reached this level only once in 10 years, yet 17 species of fish were eliminated.

In response to a question on the origin of the 4 µg/L of selenium in the uplake arm, Dr. Lemly replied that it must have come from backflow from the main lake, because he doubted that there was significant contribution from atmospheric deposition. Dr. Teresa Fan asked whether it had actually been determined that selenium was incorporated into proteins in the species with which Dr. Lemly was working. Dr. Lemly said there had been some speciation work done, but that he did not know if there were differences between mosquitofish and bluegill in terms of selenium incorporation into protein. He said that this was one possible explanation for why mosquitofish accumulate higher tissue levels of selenium than bluegills yet show fewer effects. Dr. Steven Hamilton asked about Dr. Lemly's statement that 10 µg/g of selenium in fish eggs is correlated with 5 µg/g in the food chain and 2 µg/L in the water column. Dr. Lemly replied that this statement was based on both data from the Belews recovery period and data from other lakes.

Modeling Selenium in Aquatic Ecosystems

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Dr. George Bowie of TetraTech gave a presentation entitled "Modeling Selenium in Aquatic Ecosystems," and referred to the paper "Assessing Selenium Cycling and Accumulation in Aquatic Ecosystems" (Bowie et al., 1996). (See Appendix E for presentation materials.) The model was sponsored by the Electric Power Research Institute (EPRI) and was developed in conjunction with a major research program. The research had two major components: toxicology and biogeochemical processes. Dr. Bowie's presentation focused on three of the five major components of the model: cycling processes in the water column and in the sediments, and accumulation in tissues of organisms.

For each of these areas, Dr. Bowie described the processes in the model, discussed areas of uncertainty or limitations in our understanding of these processes, and showed the results for an example application to Hyco Lake to illustrate which processes are most important. He used these results plus some of his experimental results to discuss the response times of aquatic organisms to changes in selenium exposure and the effects of water quality variables on selenium uptake. Since the model description, Hyco application, and conclusions are covered in the paper, Dr. Bowie listed the main points concerning uncertainty, pharmacokinetics, and water quality effects on uptake that are not included in the paper.

Water-Column Uncertainty:

Organic selenides represent a lumped selenium pool that includes many different selenium compounds which are poorly understood and most of which cannot be measured with current analytical techniques. Some, such as selenomethionine, may be very biologically reactive while others may be much more refractory. Most of the organic selenide pool is not selenomethionine since the high uptake rates measured in the lab are not consistent with accumulation levels and organic selenide turnover times observed in the field.

Sediment Uncertainty:

Sediment selenium accumulation depends on settling of particulate selenium (plankton, suspended organic detritus, elemental selenium, selenite adsorbed on clays), diffusion of water column inorganic selenium into sediment porewaters followed by rapid reduction to elemental selenium in anaerobic sediments, and decomposition of organic detrital selenium in the sediments. In lakes where sediments are usually anaerobic below a thin oxidized microzone, diffusion of inorganic selenium and subsequent reduction to elemental selenium is one of the most important processes. However, in other types of systems where the sediments are aerobic or anaerobic at much greater depths, other accumulation processes would be more important. Selenium speciation data in other types of systems are currently lacking, which limits an assessment of accumulation mechanisms in these systems. Sediment selenium concentrations depend not only on the selenium fluxes into the sediments, but also on the sediment deposition rates (and sediment transport rates in flowing systems). This makes sediment selenium concentrations very dependent on site-specific conditions.

Food Web Accumulation Uncertainty:

Most research on selenium accumulation in aquatic organisms has focused on planktonic food webs. Benthic invertebrates can be an important source of selenium accumulation in fish, and since the sediments contain most of the historical selenium loadings in aquatic ecosystems, detrital and sediment pathways to benthic organisms could be extremely important. Bacteria accumulate selenium to levels several times higher than algae, so sediment bacteria associated with organic detritus could be an important source of selenium accumulation in benthos. Much of the sediment selenium in lakes is elemental selenium, which was recently shown to be bioavailable to benthos (though organic selenium assimilation efficiencies are several times higher). The selenium

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concentrations in organic detrital particles, associated bacteria, and the amount of elemental selenium ingested during feeding are what determine selenium accumulation in benthos, not the selenium concentrations in the bulk sediments. Systems with high sediment deposition rates or high sediment transport rates could dilute selenium concentrations in bulk sediments, even though the selenium content of the organic food particles remained the same.

Response Rates of Organism Tissue Concentrations to Changes in Exposure:
   Uptake and depuration experiments, as well as other studies in the literature, indicate that the time it takes to reach equilibrium starting from no previous selenium exposure is on the order of a few days to a week for algae and bacteria, 1 week for microzooplankton, 1 to 2 weeks for zooplankton and benthic invertebrates, and 3 to 10 months for fish. Since most fish experiments are conducted with small fish in the laboratory, larger fish in the field could respond more slowly. Food is generally the primary route of selenium accumulation in consumer organisms, and since the sediments respond much more slowly to changes in selenium loadings than the water column, the benthic food web can continue to provide exposure to fish long after the planktonic food web levels drop.

Water Quality Effects on Selenium Accumulation:
   Since most selenium accumulation occurs at the bottom of the food web and then moves to higher trophic components through food exposure, water quality factors that influence accumulation in primary producers can be very important. In experimental research with phytoplankton, three water quality variables had a significant effect on selenium uptake rates (Riedel and Sanders, 1996). Low pH and low phosphate increased selenite uptake by a factor of about 4 or 5, and low sulphate increased selenate uptake by a factor of 2.

Dr. Fan asked Dr. Bowie if the elemental selenium data he was using for sediments involved analytical confirmation. Dr. Fan cautioned that her group could not confirm using extraction methods that the red amorphous material secreted from algae was elemental selenium; this material contained <10% Se and >90% carbonaceous material, possibly polysaccharides. She suggested a particular analytical technique that should be used for elemental selenium. Dr. Bowie replied that he was using results from Dr. Greg Cutter's work (Cutter, 1991), but that Dr. Terry Layton's work (not yet published) at the University of California at Berkeley used the analytical technique referred to by Dr. Fan and found that a significant portion of the sediment selenium was elemental selenium.

Chair's Charge to the Experts and Highlights of Premeeting Comments

Dr. Fairbrother summarized the technical charge given to the experts by EPA, and the experts' premeeting responses to the questions in the charge. (See Appendix E for presentation materials.) She noted that the leaders of each discussion session would present the premeeting comments in more detail.

Dr. Fairbrother repeated that the charge to the experts was to address and comment on technical issues. She asked the experts to identify the rationale behind their comments and conclusions, assess the level of confidence in data cited, and discuss data quality.

Dr. Fairbrother first addressed the question "What do we know about the relationship between water-column measurements of selenium and biological effects?" She said that the experts generally agreed that

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looking at this relationship alone is not a good approach for a bioaccumulative compound like selenium. Many of the experts noted that the most sensitive fully aquatic species are fish species and that diet is the primary exposure route. Also, there seemed to be a need to discuss selenium chemistry.

Next, Dr. Fairbrother discussed the experts' comments on the relationship between tissue concentrations and either sediment or water concentrations. She said that there had been mixed responses on this issue. There was disagreement on the state of the science; some of the experts said that the science base was good, while others said that there was too little data. The experts also disagreed somewhat in what form of selenium to measure in which tissue. There was some agreement that water-tissue correlations are poor, and that diet-tissue-effects correlations are better.

Concerning the link between sediment concentrations and both water concentrations and effects, Dr. Fairbrother said that there had been disagreement on several aspects of this question. Experts disagreed about the ability to relate sediment concentrations to either water-column concentrations or effects in fish. Finally, Dr. Fairbrother said that some of the cross-cutting issues brought up included selenium geochemistry, selenium kinetics within and between ecosystem compartments, and the differences between lotic and lentic systems.

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II. CHAIR'S SUMMARY OF WORKSHOP DISCUSSIONS

The following summary was written by the Workshop Chair, Anne Fairbrother, based on the experts' discussion and premeeting comments. Details of the experts' discussions are provided in Section III.

The technical sessions initiated discussions among the experts by first reviewing the questions provided in the premeeting comments and then allowing conversation to develop around a general theme. General themes were: relationship of effects to water, sediment, or tissue concentrations and a session on cross-cutting issues to capture ideas on chemistry, system variability, and other topics brought forward by individual experts.

Water-Effects Relationships

This session began with a discussion of the scientific validity of predicting chronic effects of selenium from water concentrations. The experts quickly agreed that waterborne exposure to selenium in all its various forms is less important than dietary exposure in determining the potential for chronic effects. Therefore, predictions of ecological effects cannot be based on studies that use water-only exposures. Factors that modify the relationship between water concentration and effects include the types of organisms constituting the food web, speciation and rates of transformation of selenium, and rates of exchange of selenium between water, sediment, and organisms. It was noted that selenium speciation may be sensitive to salinity, thus altering bioaccumulation potential, but this has not yet been proven.

There were differences of opinion about what to measure in the water column for assessing the level of selenium contamination of an aquatic system. However, it was agreed that, at a minimum, dissolved (i.e., in the water phase) versus particulate (i.e., attached to particles of inorganic substances or to bacteria or phytoplankton) selenium be differentiated and that selenate and selenite (two oxidation states of selenium) be determined in both fractions. Peptide- and protein-bound forms of selenium are critically related to the potential for occurrence of chronic effects. The protein-bound forms should be specifically included in the analysis of selenium in the particulate fraction, as this is the primary step for the major route of bioaccumulation. The current definition of the dissolved fraction is the portion of the sample that passes freely through a 0.4 μm filter. One expert suggested that an 0.2 μm filter might be more appropriate in order to catch the smaller phytoplankton and bacteria in the particulate fraction, as these organisms are very important in the first step of bioaccumulation of selenium.

Experts concluded that insufficient information exists to quantitatively correlate water quality characteristics (such as sulfate, pH, and TOC) with chronic toxicity. Finally, the experts emphatically agreed that toxicity relationships derived from acute toxicity studies cannot be used to predict chronic toxicity, as the dietary route of concentration and exposure is so important for selenium. This also implies that bioconcentration factors (i.e., concentration in tissues divided by concentration in water) are not appropriate for use with this compound. In summary, water concentrations are related to effects, but it is a nonlinear (and site-specific) relationship.

Tissue – Effects Relationships

Discussion then turned to technical issues associated with a tissue-based criterion. The experts agreed that tissue integrates all exposures, whether from food or water. The best tissue in which to measure selenium is fish ovaries or eggs as concentrations have been linked to reproductive effects in some species. There

was some discussion, however, that pointed out the need to develop a larger data set encompassing interspecies variability in the ovary concentration – reproductive effects relationship. If fish ovaries are not available (i.e., sampling needs to be done during the wrong time of year), then larval stages are the next-best tissue to measure as older life-stages are less sensitive to selenium effects. Liver tissue was mentioned as a third tissue for possible monitoring of residue concentrations. Muscle-plug biopsy techniques have been suggested for use with endangered species, but do not seem to correlate well with effects.

It was also pointed out that concentrations of selenium in benthic invertebrates could be measured in order to determine the potential for effects to the lower order organisms as well as to establish potential dietary exposure values for fish. Discussion highlighted the need to standardize this method, in order to be sure that sediment is removed from the organisms guts prior to measurement. A discussion ensued about the ability of selenium to alter community relationships of phytoplankton with ramifications throughout the entire food web. However, it was agreed that fish are the most sensitive to the chronic effects of selenium and therefore fish tissue continues to be the choice for a tissue-based toxicological threshold.

Further discussion centered on the form of selenium that is most appropriate to measure in tissue. To date, nearly all of the studies have measured total selenium, but it was agreed that a more accurate representation of selenium-effect relationships could be obtained through measuring protein- or peptide-bound forms of organoselenium. The incorporation of selenium into protein is the trigger for biological effects.

Finally, it may be difficult to correlate water column concentrations with tissue concentrations. There are many examples of sites where water levels are low and tissue levels are high, as a result of previous sediment loading with current reductions in water-column selenium. Sediment (and subsequent dietary) concentrations will decline over time if water levels are kept low, but there is a considerable lag from the time when water concentrations are reduced to the time when sediment concentrations reach low levels. Therefore, if the history of a site is not known, a single measurement of water and tissue (or sediment) concentrations may provide a misleading picture and inconclusive relationships.

Sediment – Effects Relationships

Sediment is the dominant sink for selenium, and sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates. The literature relating sediment-based criteria is sparse; most participants relied on three key references in their comments. A positive relationship between sedimentary selenium concentrations and effects in fish or bioaccumulation in invertebrate larvae has been shown in a few studies. However, one expert cautioned that a no-effects determination in field studies must always be tempered with an assertion that the test was powerful enough to have detected effects if they were there, albeit at low levels.

An analysis of data focusing only on fish indicates that toxic effects may occur when total sedimentary selenium concentrations exceed 4 μg/g (dry weight). Elemental and organic selenium forms predominate in sediments. The process is affected by redox conditions, and selenium tends to associate with the organic detritus. In streams, total sedimentary selenium is related to water-column concentrations through normalization to total organic carbon. It was suggested that sedimentary aluminum concentrations might be useful as a marker for inorganic sediment composition, in an effort to further separate the detrital-bound selenium from inorganic-bound forms. For accumulation in sediments of lentic systems (i.e., lakes and slow moving water), consideration of residence time and use of a mass balance approach could relate sediment selenium to waterborne selenium.

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Because waterborne selenium concentrations tend to exhibit large temporal variations, the strength of the water-to-sediment correlation is affected by the averaging period selected. The issue of spatial heterogeneity of benthic invertebrates as well as selenium deposition and speciation is very important. Other parameters that might affect the relationship of sediment concentrations and ecological effects include water retention time, volatilization rates, the type of benthic phytoplankton community, and whether or not the system is at equilibrium. Habitat selection by different types of aquatic biota and preferential feeding habits of higher organisms also modifies selenium exposure. Various experts made the points that redox potential (i.e., amount of oxygen in the system) affects selenium speciation and that improved analytical methods for sediments are needed. Two experts advocated the expansion of the use of liquid chromatography for sediment selenium analysis.

Cross-Cutting Issues

The cross-cutting session captured issues that did not fit neatly into one of the above themes, as well as other comments or ideas. Spatio-temporal variability was addressed again, as it applies to water column, sediments, and tissues, although in different scales for each. Water concentrations may change rapidly (within days), whereas fish-tissue residue and sediment concentrations take months or years to change. The rate-limiting step may be the rate of conversion of the inorganic form of selenium to the organic form, which is a function of the species of selenium in the water column and the types of microorganisms present in the sediment.

There was agreement that the type of ecosystem has a large effect on selenium cycling in the system. Lentic and lotic (fast-flowing) systems, ephemeral or perennial waterbodies, saline systems, and northern (cold) streams, may differ in response to selenium input. Retention time of carbon, rate of sediment accumulation, rates of conversion of inorganic to organic forms of selenium, and tolerances of local species all differ among these types of systems. Bacteria and phytoplankton species differ between the two ecosystem types, which may cause differences in bioaccumulation rates. Also, lentic systems have higher primary productivity. Open (rather than closed) fish populations in lotic systems make changes in recruitment more difficult to document. While there was argument about the relative importance of considering one or both of these types of systems, there was agreement that their interconnections are important.

Two methods using existing field data were suggested for differentiating non-affected sites, areas with definite effects, and sites requiring a site-specific determination of effects. The apparent effects threshold (AET) method categorizes previously studied areas based on sediment or water concentrations. The sediment/water concentration above which effects always occurred would be identified, as would the concentration below which effects never occurred. New sites with sediment/water concentrations that fall between these two values (where effects sometimes occurred or sometimes did not) would require a site-specific assessment; otherwise, the site would be categorized as affected or not. A second method is based on fish tissue concentrations as a function of water concentrations. The empirical data from field studies that exist in the literature would be used to develop the bioaccumulation correlation on a global basis. Sites where measured fish tissue concentrations were statistically significantly different from what would be predicted based on water concentrations and the global bioaccumulation factor, would require a site-specific assessment of potential effects.

It was suggested that the Aquatic Toxicity Model presented by George Bowie could be used to make *a priori* predictions of whether a concentration of selenium in water would result in effects to the fish. Site-specific input parameters include selenium input (amount, rate, and species), flow rates, water depth, and a

few other hydrological parameters as well as food-web species. The more site-specific data that are used in the model, the more likely it is to accurately predict effects.

Selenium has the potential to interact with other metals, causing either greater or lesser responses than predicted from selenium alone. Furthermore, exposure to selenium may reduce an organism's ability to respond to other environmental stresses, such as has been shown for fish similar to those found in Belews Lake that were exposed to cold temperatures during laboratory studies. These types of interactions might confound the global empirical data set relating effects to selenium concentrations in water, sediment, or food.

Selenium is a required micronutrient for both plants and animals. Therefore, there is an exposure concentration below which insufficiency effects are seen and a different concentration above which toxicity occurs. The area in between is the Optimal Effects Concentration. In general, there is at least a 10-fold difference between insufficient and toxic concentrations and, on a practical basis, it does not appear to be of particular concern in field situations. However, this issue may be important in laboratory studies where appropriate minimum concentrations of selenium must be provided to maintain colonies of test species.

Analytic methods for detection of selenium in water, sediment, or tissue are technically complex. However, due to their importance in carefully and critically describing the systems at risk, a significant amount of time was devoted to discussion of this issue. Desired minimum detection limits, sample preparation requirements, cost, and laboratory capability all affect the selection of which method to use. A detailed summary of available methods, as well as sample collection and retention procedures, is included in the report.

One expert stated that at the national level, median background concentrations of selenium in aquatic systems do not vary greatly, being at about 0.1 μg/L. However, there was disagreement on this value and particularly on the variability in background, which is dependent upon the spatial scale of the analysis as well as on site-specific geology. Methods are being developed for differentiating between natural and anthropogenic inputs of selenium into aquatic systems, but there remains a great deal of uncertainty.

Observer comments reinforced the recommendation to develop methods for setting site-specific criteria, as a universal numeric chronic criterion for selenium is highly unlikely to be predictive of effects for any particular site.

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III. TECHNICAL DISCUSSION SESSIONS

Generally, discussion leaders organized the discussions according to the questions provided in the technical charge. Each leader opened the discussion on each question by presenting an overhead summarizing the relevant premeeting comments. The following discussion session summaries include the presentation of the premeeting comments, followed by an account of the discussion for each question of the technical charge. Overall conclusions, which were written by the discussion leaders and reviewed by the other experts, are presented at the end of the discussion summary for each session.

DISCUSSION SESSION 1:
Technical Issues Associated With a Water-Column-Based Criterion

Question 1: Besides selenite and selenate, which other forms of selenium in water are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions?

Discussion leader's summary of premeeting comments:

Dr. William Adams presented his summary of the experts' premeeting comments concerning this question as follows: Selenate, selenite, seleno-cyanate, and organo-forms (seleno-methionine) are the key forms of interest. Selenate and selenite are the predominant forms derived from mining, agricultural practices, fly ash, and natural shales. Organo-selenium compounds produced from these inorganic forms are of most ecological relevance on a chronic basis; seleno-methionine is thought to be a key chemical form. Little is known, however, about environmental exposures of organo-forms, especially seleno-methionine; there is a general lack of analytical procedures for measuring organo-forms. Dr. Adams then asked the experts for any comments concerning his summary or question 1.

Discussion:

Dr. Gregory Cutter, disagreeing with the statements concerning seleno-methionine, said that free seleno-methionine is not important in water and is easy to measure. Dr. Fan expressed skepticism about the measurement of seleno-methionine, because most methods do not involve structure confirmation. She also pointed out that seleno-methionine is abundant in macromolecules and emphasized that macromolecular seleno-methionine may be important, although this hypothesis has been neither disputed nor confirmed by the literature. Dr. Cutter agreed and also stated that, based on his analysis using acid hydrolysis and ligand-exchange chromatography, the vast majority of organic selenium in unpolluted waters is peptide-bound.

Dr. Fan mentioned the possibility of the selenonium form, a cation, being present, as shown by Cooke and Bruland (1987). She added that, based on her work, salinity can drive speciation; she has found that one phytoplankton accumulates dimethyl selenonium propionate in a euryhaline environment. Dr. Cutter agreed that selenonium can be present in highly contaminated systems.

Returning to the discussion of seleno-methionine, Dr. Chapman asked whether laboratory tests using seleno-methionine are irrelevant to environmental exposures, given the small amounts of free seleno-methionine found in water. Other experts agreed that water-only exposures to seleno-methionine are of questionable relevance, but seleno-methionine may be important in food-chain transfer of selenium.

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Question 2: Which form (or combination of forms) of selenium in water are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which forms of selenium in water would you measure for correlating exposure with adverse effects in the field?) Note: Your response should include consideration of operationally defined measurements of selenium (e.g., dissolved and total recoverable selenium), in addition to individual selenium species.

Discussion leader's summary of premeeting comments:

Dr. Adams summarized the experts' premeeting comments for this question as follows: Total recoverable selenium is a useful form to measure. This would include all forms of selenium in the water except a limited amount of non-bioavailable selenium that might be tied up in the crystalline structure of suspended solids. There are no identified actual correlations between selenium forms and chronic effects. Future efforts should focus on proteinaceous forms (especially seleno-methionine). Dr. Adams then asked for the other experts' reactions to this question.

Discussion:

Dr. Fan asked for the other experts' opinions on making correlations between waterborne particulate selenium and accumulation of selenium in the food chain. She said that she had seen a couple of papers that indicated that there was a correlation (e.g., Saiki et al., 1993). Dr. Gerhardt Riedel replied that he thought that gathering data from multiple lakes would result in a correlation that was positive but would have large confidence limits.

Dr. Cutter advocated separating total recoverable selenium into the dissolved and particulate fractions, because those pools are available to different organisms. He said that this should be done by filtration using as small a pore size as possible, preferably 0.2 microns. Dr. Riedel and Dr. Adams agreed that separating the dissolved and particulate fractions is useful.

Dr. Gary Chapman raised the issue of the operational definition of dissolved selenium, which Dr. Cutter had mentioned in his premeeting comments. He asked Dr. Cutter to discuss this issue. Dr. Cutter replied that there is some work on colloidal selenium in estuaries, including a paper by Takayanagi and Wong (1984). He thinks that, based on these papers and his work, in most systems colloidal selenium represents a small fraction of "dissolved" ($\leq 0.4 \mu$m) selenium. Thus, in his opinion, 0.4 microns is not a bad filter pore size for most systems, but he advocates 0.2 microns to ensure that the smaller phytoplankton and bacteria are included in the particulate fraction. Although Dr. Riedel suggested that cross-flow filtration could be used to get down to very small size ranges, Dr. Cutter replied that this technique is laborious. Dr. Cutter and Dr. Riedel agreed that the very small size range is not that important for selenium, although it is important for some other metals. Dr. Adams concluded this discussion by pointing out that the operational definition of "dissolved" is a topic currently under debate, particularly in respect to data collection by the United States Geological Survey (USGS).

Dr. Adams asked whether the experts thought it accurate to state that no forms of selenium in water have been correlated with chronic effects; he added that the science is uncertain, but it is probably a polypeptide/protein-bound form of selenium.

Dr. Chapman asked how much of particulate selenium is actually organic and how much is bound up in a

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mineral matrix. Dr. Fan agreed that this was an important question for thinking about bioavailability. Dr. Cutter agreed and listed the possible forms of particulate selenium: adsorbed selenate or selenite (probably on clays), elemental selenium, and organic forms. He said that Luoma et al. (1992) have looked at the speciation of selenium on particles. Dr. Fairbrother responded that the separation of organic from mineralized selenium needs further research. Dr. Fan suggested that standard biochemical procedures could be used to determine what fraction of particulate selenium is bound to proteins. Dr. Adams observed that most of the previous discussion related to possible areas of future research, rather than currently practical techniques.

Dr. Joseph Skorupa asked the biochemists present if they felt that any form of selenium was toxicologically unimportant. Dr. Fan and Dr. Cutter responded that they did not, because all forms of selenium may eventually interconvert.

Question 3A: In priority order, which water quality characteristics (e.g., pH, TOC, sulfate, interactions with other metals such as mercury) are most important in affecting the chronic toxicity and bioaccumulation of selenium to freshwater aquatic life under environmentally realistic exposure conditions?

Discussion leader's summary of premeeting comments:

Dr. Adams summarized the experts' premeeting comments for this question as follows: It is not possible to rank these water quality characteristics with reasonable certainty due to insufficient information on their effects on expression of chronic toxicity. Overall, the Eh (oxidative/reductive) state of an ecosystem is most important in determining the potential for chronic toxicity to occur, because it significantly influences the formation of organo-forms of selenium. One could predict that, at the extremes and as a function of Eh, pH would be important due to speciation changes, but chronic data are not available to assess this. pH would be expected to have the most impact on selenite across typical environmental pH values. Sulfate appears unimportant in terms of the expression of chronic toxicity except potentially for primary producers. Arsenic and molybdenum are also mobilized under similar conditions as selenium and appear to be additive with selenate.

Discussion:

Dr. Cutter agreed that redox state is important for precipitating elemental selenium and removing dissolved selenium. He argued, however, that photosynthesis has more influence on the formation of organo-selenium. Dr. Adams and Dr. Fan pointed out that non-photosynthetic microbial processes are also important, particularly in sediments; these processes are somewhat coupled to redox state.

Dr. Fan added that the presence of sulfate or nitrate in a reducing environment encourages a certain type of microbial community (sulfate or nitrate reducers), which would have a major impact on selenium speciation. She cited evidence of hydrogen selenide and methaneselenol release into the marine atmosphere via phytoplankton activities (Amoroux and Donard, 1996). Dr. Cutter expressed skepticism about this possibility. Dr. Fan, Dr. Cutter, and Dr. Adams did agree, however, that the microbial loop is very important and that the presence of sulfate and nitrate reducers would affect selenium speciation, resulting primarily in the reduction of selenium to the elemental form.

Dr. Cutter commented that arsenic and molybdenum behave differently from selenium; in a reducing

environment, arsenic is mobilized while selenium is immobilized.

Question 3B: Of these, which have been (or can be) quantitatively related to selenium chronic toxicity or bioaccumulation in aquatic organisms? How strong and robust are these relationships?

Discussion leader's summary of premeeting comments:

Dr. Adams summarized the experts' premeeting comments for this question as follows: Insufficient information exists to quantitatively correlate water quality characteristics with chronic toxicity across multiple species and trophic levels. Sulfate, phosphate, and temperature have been shown to correlate with selenate for some species (i.e., primary producers).

Discussion:

Dr. Riedel amended Dr. Adams's comment by saying that, for primary producers, phosphate does not affect selenate uptake, but rather high phosphate concentrations appear to suppress selenite uptake.

Question 3C: How certain are applications of toxicity relationships derived from acute toxicity and water quality characteristics to chronic toxicity situations in the field?

Discussion leader's summary of premeeting comments:

Dr. Adams summarized the experts' premeeting comments for this question as follows: The applications of relationships derived from acute toxicity and water quality characteristics do not apply to chronic toxicity for most aquatic life (an exception to this might be the relationship between selenate and sulfate for algae). The primary reason for this is that acute toxicity is most often the result of water exposures, whereas chronic effects are the result of selenium being incorporated into the diet where the predominant form of selenium is no longer an inorganic form.

Discussion:

None of the experts had any objections to this summation.

General Comments:

Discussion leader's summary of premeeting comments:

Dr. Adams offered for discussion the following statements taken from various premeeting comments: 1) Laboratory studies provide reasonable estimates of acute toxicity. 2) It seems imperative that chronic criteria include consideration of tissue residue and dietary route of uptake. 3) Fish eggs may represent a reasonably sensitive tissue to use as an endpoint for assessing the potential for species-level risk. 4) A useful approach might be to develop a generic criterion which also allows for site-specific approaches. Toxicity and bioconcentration factors (BCFs) are a function of time and exposure level. 5) Organic forms are thought to be produced in response to inorganic selenium enrichment and probably represent a net

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reduction in potential for toxicity.

Discussion:

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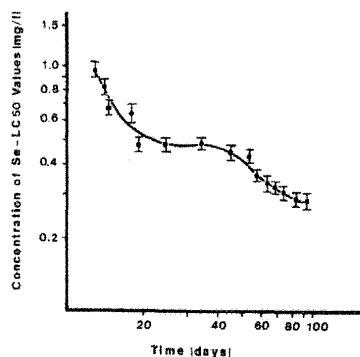

Figure 2. The effect of time on the toxicity of sodium selenite to fingerling rainbow trout. The line was fitted by eye. (Adams, 1976.)

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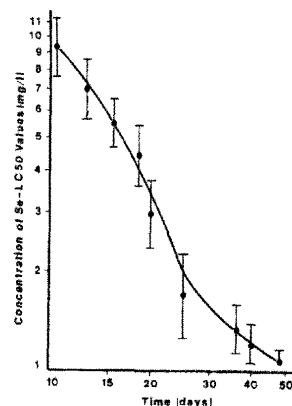

Figure 3. The effect of time on the toxicity of sodium selenite to juvenile fathead minnows. The line was fitted by eye. (Adams, 1976.)

e rates are slow), he postulated that the 96-hour assay may not be the right test for acute toxicity. Dr. Cutter questioned the relevance of a water-only exposure. Dr. Skorupa pointed out that a short-term spike in selenium may have long-lasting food-chain implications, as shown by a paper by Maier et al. (1998). In this paper, a short-term 10 μg/L spike in a Sierra Nevada stream resulted in a concentration of 4 μg/g in the food chain for over a year. Dr. Chapman replied that a tissue-based criterion would require modeling with rate and fate functions and that in such a situation there would be no reason to draw an arbitrary timeline to separate acute dosings from chronic effects. Dr. Fairbrother said that that issue would be addressed in the discussion of averaging times during the cross-cutting session.

Dr. Adams then initiated point, concerning organic pointed out that toxic and can volatilize out they can also Cutter stated that a paper showed that dissolved less bioavailable to primary forms, such as selenite. distinction between essentially nontoxic to selenate, which is agreed that concentrations real waters are probably selenate. Dr. Fan pointed organic forms may be organisms such as small

Figure 4. The accumulation of selenium in the muscle of adult fathead minnows. (Adams, 1976.)

ingest them; Dr. Cutter agreed. Overall, however, Dr. Riedel and Dr. Cutter both stated that dissolved (not particulate) organic selenium in most waters is probably fairly persistent and refractory, and not very bioavailable. (It is taken up poorly and broken down slowly.) Dr. Cutter referred to a paper his group has published, which looks at the lifetime of dissolved organic selenium in the North Atlantic (Cutter and
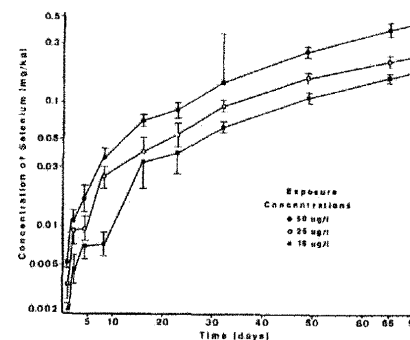
discussion on the last selenium forms. Dr. Fan methylated forms are less of the system, but that bioaccumulate. Dr. by Gobler et al. (1997) organic selenium was producers than inorganic. Dr. Riedel made the selenite, which is phytoplankton, and moderately toxic. He of organic selenium in less toxic to algae than out that particulate more bioavailable to protozoans, which can

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Cutter, 1998).

Dr. Adams directed the experts' attention to the comment concerning bioconcentration factors, which he defined as not including diet. (Bioaccumulation factors would include diet.) He showed a graph of bioconcentration factors observed at various intervals for fathead minnows exposed to four concentrations of selenium (Figure 4). Dr. Adams argued that, because there is a body of literature showing (as did his data) that BCF is inversely related to water concentration for selenium and many other metals, reporting a BCF for a given species at a given site is of questionable value. Dr. Chapman replied that he thought the experts could agree that BCFs were not relevant for selenium, as food chain is the key; Dr. Cutter agreed and said that this point should be emphasized.

Dr. Fan remarked that the emphasis on water-column concentration has led mitigators to focus on driving down those concentrations, which is not in fact the aspect of the system that is directly correlated with ecosystem effects. Dr. Fairbrother replied that EPA is struggling with this issue, because water quality criteria have been set using water column numbers. Dr. Adams postulated that the mass of selenium in the sediments may be more important than the concentration of selenium in the water. Dr. Cutter replied that water concentrations are related to effects but that it is a nonlinear relationship. Dr. Fan gave an example of two agricultural drainage ponds she has studied. Water concentrations of selenium differ by an order of magnitude between the two ponds, but sediment concentrations are similar. Dr. Adams speculated that one site might have more volatilization, and Dr. Fan agreed. Some of the experts discussed volatilization. Dr. Adams said he had seen papers that found that volatilization increases in reservoirs which have alternating drawdown and refill cycles (Hansen et al., 1998; Frankenberger and Karlson, 1994). The experts discussed the residence time of volatilized selenium in the atmosphere; Dr. Cutter said that it lasts a day or two at most, although Dr. Fan said it could be longer if the selenium attaches to particles and/or aerosols.

Dr. Skorupa asked if the apparent lack of correlation between water and sediment selenium concentrations in Dr. Fan's evaporation ponds could be due to sediment heterogeneity and small sampling size. Dr. Fairbrother replied that this question could be discussed during the sediment session.

Wrap-Up

Dr. Adams summarized the discussion session as follows: Dietary uptake is critical to determining chronic effects. The incorporation of waterborne selenium into the diet is key; factors that should be taken into account include transformations, rates of transformation, chemical species, and types of organisms (e.g., microbes, invertebrates). Peptide/protein-bound forms are important. Free seleno-methionine is typically nonexistent or at low levels.
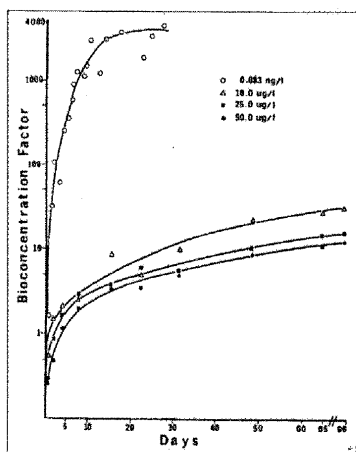

Figure 5. A comparison of the bioconcentration factors observed at various intervals for fathead minnows exposed to four concentrations of selenium. (Adams, 1976.)

Dr. Adams asked what form(s) of selenium in water should be measured relative to assessing chronic toxicity and water quality standard compliance. Dr. Cutter said that, at a minimum, selenite, selenate, and total dissolved selenium should be measured. Another expert added that particulate should be measured as well. The experts discussed this question but did not come to agreement. Experts with opinions on this topic were asked to write summaries of their opinions.

Dr. Fan gave the following summary of her opinion regarding the significance of differentiating the protein-bound fraction of particulate selenium in the water column:

> Particulate selenium can originate from live planktonic organisms, organismal debris/waste, and soil/sediment particles. The bioavailability of selenium associated with these different sources can vary. Presumably, selenium associated with organisms and biodebris represents a dietary route of exposure for aquatic consumers, and this fraction of selenium may be more concentrated and bioavailable. Since selenium bioaccumulation and toxic effects are mainly expressed through dietary exposure, it is important to distinguish the fraction of particulate selenium that is more representative of the consumers' diets. However, it would be a difficult task to speciate all of the selenium in particulate matter that is of biological origin. The fraction of biogenic selenium associated with soluble proteins may be convenient, because it may also be the most significant selenium sink in planktonic organisms exposed to environmentally relevant waterborne selenium concentrations. Major incorporations of selenium into bulk algal proteins have been documented for several categories of algae (Wrench, 1978; Fan et al., in press; Fan et al., 1998). Based on known selenium biochemistry (e.g., the propensity of selenium to substitute in sulfur amino acids), similar incorporations may well be applicable to other planktonic organisms. Therefore, monitoring protein-bound selenium in particulate matter may provide a more representative linkage from water to aquatic consumers in terms of selenium exposure.

Dr. Adams gave the following summary of his opinion regarding total recoverable selenium measurements:

> Total recoverable selenium is recommended as one of several measurements that could be made to correlate with adverse effects in the field. This measurement includes all of the forms of selenium present in a water sample (both dissolved and particulate) except those tied-up in the crystalline structure of suspended solids. This recommendation is based on the need to identify a measurement that can be performed routinely and reliably across multiple laboratories. Additionally, many of the existing relationships between water, sediment and tissue have been developed around either total recoverable selenium or dissolved selenium. Ultimately, what form(s) of selenium should be measured depends upon the use of the data.

Dr. Cutter gave the following summary of his opinion regarding selenium measurements:

> Additional measurements that are recommended for water include dissolved (defined as ≤ 0.4 μm) and particulate selenium. Dissolved measurements would be measured as total dissolved selenium, selenate, and selenite. Se^{-2} (selenides) would be determined by subtracting $Se^{+4} + Se^{+6}$ from total dissolved selenium (Cutter 1982). Particulate selenium (defined as selenium associated with particles >0.4 μm) could be measured as total selenium as well as Se^{+4} and Se^{+6}. Elemental selenium would be determined separately by direct analysis for Se^0 (Velinsky and Cutter 1990). Se^{-2} would be determined by difference (i.e., subtracting [elemental + Se^{+4} + Se^{+6}] from total particulate selenium). As an approach to reduce costs one could consider speciating samples, especially the particulate

fraction, only on a periodic basis.

Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.

1.   Waterborne exposure to selenium in all its various forms is much less important than dietary exposure in determining the potential for chronic effects in aquatic organisms in general and for fish in particular.

2.   The relationship between selenium in water and sediment relative to the aquatic organisms that live in these compartments and constitute the diet of fishes is key to understanding the food chain transfer of selenium. Factors that are important in understanding these relationships include rates of transformation and speciation of selenium, rates of exchange of selenium between sediment and water and organism tissues, and types of organisms constituting the food web.

3.   Peptide- and protein-bound forms of selenium in the diet of aquatic organisms are emerging as critical factors in assessing the potential for chronic effects in aquatic organisms. Free seleno-methionine appears to exist only at very low levels in tissues and in water.

4.   Bioconcentration and bioaccumulation factors are inversely related to water exposure levels, which complicates their use in developing water quality criteria.

5.   To evaluate selenium in the water compartment of aquatic ecosystems it is recommended that at a minimum dissolved versus particulate selenium be differentiated and that selenate and selenite be determined in the dissolved fraction. Additionally, it appears useful to determine selenite, selenate, and protein-bound and total selenium in the particulate fraction of natural surface waters. The latter may be of less importance for industrial discharges.

DISCUSSION SESSION 2:
Technical Issues Associated With a Tissue-Based Chronic Criterion

Dr. Hamilton opened the session by remarking that tissues integrate all exposures an organism experiences and represent the biological effects that water quality criteria are intended to prevent.

Question 4: Which forms of selenium in tissues are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions and why?

Discussion leader's summary of premeeting comments:

Dr. Hamilton presented a brief summary of each individual's comments on this question. He said there was general agreement that the form of selenium of concern in tissues was an organic, or protein-bound, form. He asked for any comments or concerns.

Dr. Chapman asked whether this question included organisms fed on by fish, pointing out that, if so, it would be important to think about the issue of gut contents and to specify whether organisms should be

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depurated. Dr. Fairbrother asked the other experts to clarify whether fish were the only organisms in which effects were to be discussed, or whether anyone would say that selenium affects other organisms. Dr. Fan replied that, based on her review of the literature, there are not mortality or direct toxic effects on phytoplankton or invertebrates, but there may be community change. Dr. Riedel agreed. Dr. Fan and Dr. Riedel submitted additional comments on this point.

Dr. Fan submitted the following comments on the potential effect of selenium on community structure:

It is clear that selenium, regardless of the form, is less toxic to lower trophic organisms including primary and secondary producers, zooplankton, and benthic invertebrates. Selenium contamination, however, can have an effect on the competitiveness of different components of a given community, leading to an alteration of the community structure. For example, in San Francisco Bay in the 1980s, a shift from a diatom-dominated to a green algal community occurred. This shift preceded an explosive growth of the Asian clam, *Potamocorbula amurensis*, which is an extremely efficient accumulator of selenium (Brown and Luoma, 1995). It is unclear whether selenium contamination contributed to the change in the algal community, nor can we draw conclusions about the role of selenium in the abundance of the Asian clam. However, selenium is interacting with this new trophic system, and a selenium bioaccumulation factor of over 100,000 from water to the clam has been observed. In addition, the Asian clam is an important food source for the indigenous sturgeon. There is some evidence that the sturgeon population in the Bay is not actively reproducing and that field-collected sturgeon eggs exhibit high parts per million (ppm) selenium concentrations, particularly in certain protein fractions (Kroll and Doroshov, 1991). Unfortunately, the relationship between high selenium egg content and sturgeon reproduction problems has not been clearly established. It remains a real possibility, however, that selenium plays an important role in the impact of altered lower trophic community structure on fish reproduction.

Dr. Riedel submitted the following comments on selenium toxicity and algal communities:

Although most of the discussion of selenium toxicity has focused on fish reproductive effects, selenium toxicity can exert other effects on aquatic ecosystems. In some cases, environmental concentrations of selenium can also exceed the acute toxicity thresholds for a variety of algal species. The toxicity of selenium to algae is dependent both on the species of algae and the form of selenium. Of the two predominant forms of inorganic selenium in water, selenate has been generally observed to be more toxic to algae than selenite. For example, selenate concentrations from 50 to greater than >10,000 µg Se/L have been observed to inhibit growth of three species of phytoplankton from three different taxa. A diatom, *Cyclotella meneghiania*, was observed to be the most sensitive (EC_{50} ≈ 200 µg/L). A green alga, *Chlamydomonas reinhardtii*, was the next most sensitive (EC_{50} ≈ 2,000 µg/L), while the cyanophyte *Anabaena flos-aquae* was the least sensitive, with an EC_{50} of >10,000 µg/L. None of these species were inhibited by concentrations of selenite up to 10,000 µg/L (Sanders et al., 1989). Similar toxicity results have been reported by Wheeler et al. (1982). Other authors, notably Kumar and Prakash (1971) and Moede et al. (1980), have observed that selenate and selenite have similar effects on several algal species. At least one green algae, *Ankistrodesmus falcatus*, may be unusually sensitive to selenite; Dr. Riedel has observed near complete growth inhibition in cultures spiked with 10 µg/L selenite, but not selenate (Riedel, unpublished observation).

Dr. Riedel has observed at least one "field" case of selenium toxicity at concentrations representative

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of mildly contaminated sites. Riedel et al. (1996) made 10 µg/L additions of both selenate and selenite to natural phytoplankton cultures collected from Hyco Lake, as part of a biotransformation experiment. The selenate cultures showed a mild reduction in growth rate and maximum yield (~10%) compared to the control and selenite cultures. To verify the study, a series of selenate and selenite additions were made to another natural collection from the same site one month later; in this case, 10 µg/L selenate showed no inhibition, 20 µg/L decreased growth more than 10%, and inhibition was complete at 200 µg/L. Selenite did not show inhibition in these experiments either.

If selenium toxicity to a particular species or group of species were to occur in the field, it would be very difficult to observe from the existing community; the absence of some subset of possible species would not readily be detected (unlike the situation of fish in Belews where some 13 of 17 possible fish species were eliminated, there are hundreds of possible phytoplankton species, and rapid changes in species composition is the norm). Even a relatively small decrease in growth rate by an individual species could lead to a very rapid decline in its abundance relative to unaffected species. Nevertheless, the lack of these species could be significant in the food web, or as links in the chain of selenium bioaccumulation and biotransformation. If the sensitive species are truly randomly distributed among taxa, size classes, edibility to higher trophic levels, etc., differential selenium toxicity to phytoplankton is probably not a significant influence on aquatic ecosystems. It is unlikely, however, that the effects are truly random, and the net effect of selenium toxicity to phytoplankton may be to inhibit large cells to a greater extent than small cells (e.g., Munwar et al. 1987), diatoms to a greater extent than blue-greens (e.g., Sanders et al., 1989), and so on.

To return to the original question about toxicologically important selenium forms in tissue, Dr. Fan said that she did not believe that all selenium in tissue is in the protein-bound form. She cited a study of her group's, currently in press, which found that the percent allocation of selenium into protein in algae varies with varying selenium concentration (Fan et al., in press). Dr. Cutter, referencing his dissertation work (Cutter, 1982), said that the remaining selenium could be going into selenium esters, found in membranes. Dr. Hamilton asked the experts whether the bottom line of the discussion was still that incorporation of selenium into protein was the trigger for biological effects. The other experts agreed that this is at least "a" bottom line.

Question 5: Which form (or combination of forms) of selenium in tissues are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which forms of selenium in tissues would you measure for correlating exposure with adverse effects in the field?)

Discussion leader's summary of premeeting comments:

Dr. Hamilton summarized the experts' premeeting comments for this question as follows: There were a variety of answers and agreement on some points. The experts agreed that there has been little speciation work in fish tissue. The forms suggested for measurement were largely total selenium or protein-bound selenium. William Van Derveer said that he would measure total selenium only if the exposure was a field exposure.

Discussion:

Dr. Hamilton asked Mr. Van Derveer to elaborate on his premeeting comments. Mr. Van Derveer replied that his concern is that, in laboratory studies, when diets are dosed with a specific selenium form, the residues that accumulate in the tissues may differ from the full biogeochemical spectrum that is found in the field. Dr. Hamilton replied that he had done a study in which fish were fed diets either spiked with seleno-methionine or made up of selenium-contaminated organisms from the field. He found mirror-image effects between the two diets (Hamilton et al., 1990). He added that there has been at least one other study that indicated that seleno-methionine is a good model for selenium present in the food chain (Bryson et al., 1985). Dr. Skorupa said that there is fairly strong consensus in the scientific literature that food-chain selenium, even though it is derived from different forms in water, exerts the same toxicity on a gram per gram basis. Besser et al. (1993) showed that seleno-methionine, selenate, and selenite bioaccumulate to different levels, but exert the same toxicity at the same levels. However, the various forms will move differently from water into the food chain; for example, compare Chevron Marsh to Kesterson (Skorupa, 1998). Dr. Cutter pointed out that the Bryson et al. study related to water exposure, not selenium added to the diet.

Dr. Hamilton summarized that the form of selenium in the tissue most closely associated with biological effects is an organic form. Dr. Fairbrother reminded the other experts that the original question was what to measure in tissues. She added that, historically, total selenium is what has been measured in tissues to relate to effects, but that in the future more measurement of protein-bound selenium should be done. Dr. Hamilton agreed, but Dr. Riedel said that, from a monitoring perspective, total selenium is adequate for tissues. Dr. Fairbrother pointed out that the morning's discussion indicated that there is not always a good correlation between total concentrations and effects. She speculated that these differences could be related to different amounts, or different types, of protein-bound selenium. The experts discussed the implications of the variation in the correlation between tissue levels of selenium and effects. Some argued that this variation mostly results from individual and interspecies variation in metabolism and fitness, whereas others said it may result from different forms of selenium in the tissues. The latter group thus argued for improved speciation of selenium forms in tissue.

Question 6: Which tissues (and in which species of aquatic organisms) are best correlated with overall chronic toxicological effect thresholds for selenium?

Discussion leader's summary of premeeting comments:

Dr. Hamilton summarized the experts' premeeting comments as follows: Almost all of the experts said that reproductive tissue is best correlated with effect thresholds. Some suggested that whole-body residue measurements would also be acceptable; whole fish are easier to obtain and much of the data in the literature is on whole-body residues. Dr. Fairbrother and Dr. Chapman suggested sampling benthic invertebrates; Dr. Cutter recommended the cytosol fraction of prey organisms.

Discussion:

Dr. Hamilton asked the experts whether they could recommend the ovaries as the tissue of choice, even though ovaries are not available all year. After a brief discussion, the experts agreed that fish ovaries are the tissue of choice in which to measure selenium levels. This agreement, however, was followed by further discussion.

Dr. Adams said that there needs to be a great deal more data on the variability of thresholds of effect

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among various species, habitat types, and environments. Dr. Hamilton agreed. Dr. Adams said that it would be important to characterize the distribution of sensitivity among organisms of interest, as is currently done for the water-column criteria. Dr. Fairbrother asked whether the variability is based mostly on species sensitivity, or whether the type of selenium measured and the problem of gut contents contribute to the variability. Dr. Hamilton said that a lot of the variability in the current data set is due to life stage, as older organisms are more resistant. He said that, if whole-body residues are used, larval fish should be sampled.

Dr. Fairbrother asked Dr. Skorupa to comment based on his experience with the agricultural drainwater study. He replied that that type of dataset would be useful for taking a probabilistic approach to the criterion. The National Irrigation Water Quality Program (NIWQP) dataset (Seiler, 1996) has a large amount of data relating water concentrations to fish tissue levels (almost exclusively whole-body). Dr. Skorupa said that this data could be used, along with good measures of tissue effect levels, to develop a water column number that was associated with a certain probability of exceedance of effect thresholds. He agreed that more work would need to be done on effect-level variability among species. Dr. Fairbrother said that, if this type of analysis were done, it would be important to look at all the relevant parameters, such as what type of selenium is measured, whether the gut content is included, etc.

Dr. Fan asked how endangered species could be sampled for regulatory purposes. Dr. Hamilton replied that a muscle-plug technique has been developed, in which a biopsy is analyzed by neutron activation. Unfortunately, muscle tissue does not seem to correlate well with effects, based on his research (Hamilton, unpublished). Dr. Fan asked if blood sampling is an option; Dr. Riedel replied that it is, although it is hard to get blood from the smaller fish. Dr. Hamilton said that he has seen sampling of gills, blood, heart, and liver, but that are few data on these tissues. Dr. Riedel responded that his group had sampled various tissues in fathead minnows. They found that selenium concentrations increased more slowly in muscle tissues than in other tissues. Selenium concentrations in livers, however, mirrored concentrations in ovaries (Dr. Denise Breitburg, unpublished research for the EPRI project). Dr. Riedel noted that, unlike ovaries, livers are available all year.

Dr. Adams said that he thinks gonadal tissue is by far the first choice, because it is where the most sensitive effect is expressed; it is worth waiting to sample this tissue when it is available. Other experts agreed, although it was pointed out that there are additional sampling difficulties; some fish bear their young live, and sometimes it is difficult to get gonadal tissue even during the reproductive season. Dr. Lemly said a good approach would be to target a sensitive species that is widespread, such as a salmonid or a centrarchid, depending on the water body. Other experts reiterated that assessing data sensitivity across species would be crucial to the establishment of a tissue-based criterion.

Question 7: How certain are we in relating water-column concentrations of selenium to tissue-residue concentrations in top trophic-level organisms such as fish? What are the primary sources of uncertainty in this extrapolation?

Discussion leader's summary of premeeting comments:

Dr. Hamilton summarized the experts' premeeting comments as follows: Experts expressed that they were "not very certain" about making these correlations.

Discussion:

Dr. Hamilton made the point that there are many situations in which the water-column concentration of selenium is low but tissue levels are high (Hamilton et al., 1990; Schroeder et al., 1988; Skorupa and Ohlendorf, 1991; Zhang and Moore, 1996). Loading to tissue can come from the sediments and biota as well as from the water. Dr. Hamilton also asked whether it is possible that seleno-methionine is found in such low concentrations in the water column because it is highly bioavailable and taken up immediately when cells lyse. Dr. Cutter said that his group is working on this question.

The experts discussed using the NIWQP dataset to develop an empirical probabilistic approach to correlating water-column to tissue concentrations of selenium. Dr. Adams did not have great success in an initial attempt to make these correlations (Adams, unpublished), but he plans to redo his analysis. Dr. Hamilton said that better correlations could probably be achieved by taking site-specific factors into account. Dr. Adams agreed; he said that some of the published studies say that selenium transfer from the water to the food chain can be predicted well within a small site, but attempts to extrapolate to a regional or national scale fall apart.

Dr. Cutter raised the issue of detection limits, which he said are often not low enough for researchers to adequately make the correlations that are attempted. He recommends 0.01 ppb, because most uncontaminated waters are below 0.1 ppb total selenium. He and Dr. Skorupa discussed this issue. Dr. Skorupa questioned whether such a low detection limit is necessary if the effects threshold is much higher. Dr. Cutter responded that the lower the detection limit, the more useful the data will be for future uses and for looking at sublethal effects. Dr. Fairbrother agreed that a low detection limit was a good idea when trying to establish water-tissue correlations. Some experts objected to the characterization of the natural background concentration of selenium as 0.1 ppb, but this discussion was tabled until the cross-cutting session.

Dr. Hamilton then asked whether the other experts thought there would be more certainty in relating dietary concentrations to tissue residue in fish, and then in the two-step process of relating water to food organisms to fish. The experts agreed that there would be more certainty in these relationships, but that they still would be difficult to quantify. Many of the experts mentioned the difficulty caused by spatial and temporal variability in water-column selenium concentrations. Dr. Fan also questioned how to define diet. She mentioned Saiki's work in the San Joaquin River and San Luis drain (Saiki and Lowe, 1987; Saiki et al., 1993), which showed a good correlation between benthic invertebrates and detrital selenium. She emphasized, however, that it is crucial to determine what organisms are actually eating when trying to model food-chain transfer. Dr. Hamilton added that this point brought up the issue of sediments, which can be a source of loading to the food chain, and thus should potentially be included in correlation models. Dr. Fan said that migration of organisms in and out of the system poses another problem for correlations.

Wrap-Up:

Dr. Hamilton summarized the discussion from this session. He said that he thought the experts had come to agreement that tissue integrates all exposures, whether different food types or water. Issues that had been raised included community change and variability in the sensitivity of the reproduction endpoint across fish species, and sometimes within species; there are limited data on both of these topics. He said that the group had not thoroughly discussed which endpoint was appropriate to examine (e.g., mortality, growth, deformities). Dr. Fan responded that this is why she thought the blood idea would be interesting. Selenium may reduce blood's oxygen-carrying capacity, and this endpoint would respond fairly quickly to ingestion of selenium. Dr. Hamilton replied that an important question to ask in considering an endpoint is whether

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the effect is reversible. If so, the effect may not be truly adverse; it may not have effects at the population level.

Dr. Hamilton said that the experts had largely agreed that the ovary is the best tissue in which to measure residues; larval fish are a second choice if ovaries are not available. He reiterated that the issue of sensitive species is key. He said that information on linking sediments or water back to tissue is a data gap; too few data exist to build a good model. Dr. Adams said that he thinks the data exist, but that gathering sufficient data to encompass variability within and across sites would be a large task. He added that EPA should make a broad effort to compile these data sets. Dr. Fairbrother put in a cautionary note that the empirical approach of using large data sets to look at correlations is a useful starting point, but the real goal should be to understand mechanistically how selenium moves through the different compartments in different systems. Dr. Hamilton agreed, and said the data set should be built around reproductive studies in a series of fish species.

Dr. Hamilton said that some of the experts had suggested sampling benthic invertebrates because they are a key component of the food chain. He agreed that this is a good idea, and added that tissue concentrations in these organisms will be less variable than other components of the ecosystem. Dr. Riedel pointed out that selenium concentrations in benthic invertebrates are highly affected by gut contents, but other experts replied that this problem can be solved by depurating the organisms. Dr. Adams said that which compartment is most variable can be site-specific; sediments can be very heterogeneous and may therefore be highly variable. Other experts responded that this problem could be addressed by sampling in multiple locations.

Dr. Adams made the final point that, when looking at sensitive species, it is important to look at species that actually occur in the region under study. Dr. Hamilton agreed and added that, in the west, one may want to differentiate between native and introduced species.

Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.

There was an unexpected, readily reached agreement on the four issues concerning the possibility of a tissue-based chronic criterion. The experts agreed that the selenium form in tissue that is toxicologically important with respect to causing effects on freshwater aquatic organisms under environmentally realistic conditions is protein-bound selenium. By "protein-bound," experts meant all organic selenium forms as a group. It was acknowledged that different forms of selenium can exist in tissue, but analysis of tissue selenium is typically as total selenium and not by speciated forms. In general, the organisms of concern were fish, which is the group usually emphasized in consideration of adverse effects on aquatic life. However, aquatic invertebrates were mentioned as another tissue of concern, because they represent an important link in food-chain transfer of selenium in the aquatic environment.

Protein-bound selenium, measured as total selenium, is the selenium form related to chronic toxicity. The major concern was organo-selenium forms bound by proteins rather than free organo-selenium or inorganic forms. One concern raised was that the form of selenium to which organisms are exposed might influence the resulting tissue residue; thus, emphasis should be on use of data from environmental field studies rather than laboratory studies in establishing a tissue-based criterion. The key tissues identified by experts were fish gonads, ovaries, or eggs. Due to the limited availability of ripe gonads/eggs, however, newly hatched larvae analyzed for whole-body residues were recognized as a possible alternative. Most data are on

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whole-body fish, but for a variety of life stages rather than the preferred, sensitive larval life stage. The dataset for gonads, ovaries, and eggs are more limited. Liver tissue was mentioned as a third tissue for possible monitoring of residue concentrations.

Referring back to the dietary route for selenium, benthic invertebrates were recognized as a possible group of organisms to monitor in assessing adverse effects on aquatic environments, especially from the standpoint of shifts in the composition of a community and the resultant effects on higher trophic levels which might also shift in composition. One concern with benthic invertebrates was possible errors in residue concentrations due to gut contents.

Even though tissues were readily embraced as a possible component for establishing a criterion for selenium, the relation to water concentrations was questionable. Experts readily acknowledged that there was a lot of uncertainty in modeling the relation between concentrations in fish tissue and water. However, the level of uncertainty was less for the relation of selenium in water to that in aquatic invertebrates, and concomitantly, from selenium in dietary organisms to fish tissue.

Data gaps were identified including the limited number of fish reproductive studies where exposures included water and dietary routes using realistic water characteristics and food organisms and where meaningful endpoints were measured such as egg and larvae residues along with biological effects on offspring. These reproductive fish studies should include several representative families of fish.

DISCUSSION SESSION 3:
Technical Issues Associated With a Sediment-Based Chronic Criterion

Mr. Van Derveer opened the session by making some general observations based on the premeeting comments. First, sediment is the dominant sink for selenium. Second, sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates, and selenium tends to accumulate in detritus. He added that the literature applicable to sediment-based criteria is sparse; most participants relied on two to three references in their comments. Finally, he said that there was a range of opinions expressed in the comments regarding the potential merit of a sediment-based criterion.

Question 8: Which forms of selenium in sediments are toxicologically important with respect to causing adverse effects on freshwater aquatic organisms under environmentally realistic conditions?

Discussion leader's summary of premeeting comments:

Mr. Van Derveer presented a brief summary of each individual's comments on this question. Experts expressed a range of different opinions. Forms suggested included total selenium, elemental and organic selenium, and detrital selenium. Various experts made the points that redox affects speciation and that improved analytical methods are needed.

Discussion:

The issue of sediment heterogeneity was raised and discussed by some of the experts. They agreed that selenium can be distributed very heterogeneically in sediments, and that this should be considered in sampling and modeling. Dr. Skorupa added that the spatial heterogeneity of benthic invertebrate

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distribution should also be noted. He said that this distribution often maps onto the spatial heterogeneity of selenium; both are found in areas of fine organic matter. In his opinion, sampling that does not concentrate on these areas misrepresents the toxicological risk. Dr. Riedel agreed and said that normalization to total organic carbon (TOC) is one way to solve this problem. Mr. Van Derveer said that he would later present some data showing that depositional zone selenium concentrations can fairly well predict concentrations in riffle-dwelling midges.

Mr. Van Derveer asked Dr. Adams to elaborate on his call for improved analytical methods for sedimentary selenium. Dr. Adams replied that he sees variability among analytical laboratories in determining sediment selenium speciation. Dr. Cutter responded that the techniques are established, but that better training may be needed. Dr. Skorupa said that he agreed with Dr. Adams, and added that it is important that all analytical data be evaluated. Dr. Riedel agreed that there is a problem with analysis for selenate. He and Dr. Fan advocated the expansion of the use of liquid chromatography for selenium analysis.

Mr. Van Derveer asked if there were any other issues related to question 8, recognizing that the literature relating sediment concentrations to toxicity is sparse. Dr. Cutter replied that, because of the lack of literature, the conclusion should be that the experts had low confidence in answering the question; Dr. Riedel agreed.

Mr. Van Derveer presented a graph using data from a publication of his (Van Derveer and Canton, 1997) (Figure 5). The graph showed the relationship between sedimentary selenium concentration and effects in fish, using data from a variety of sources, including NIWQP, Belews Lake, Hyco, and others. Mr. Van Derveer said that there appears to be a clear concentration-response ratio, but that more data are needed. Dr. Skorupa cautioned that the power of the study should be kept in mind when there is a finding of "no effect," as many studies lack the necessary power to detect effects.

Question 9: Which form (or combination of forms) in sediment are most closely correlated with chronic effects on aquatic life in the field? (In other words, given current or emerging analytical techniques, which forms of selenium in sediments would you measure for correlating exposure with adverse effects in the field?)

Discussion leader's summary of premeeting comments:

Mr. Van Derveer presented a brief summary of each individual's comments on this question as follows: He himself said to measure total selenium and

Figure 6. Reanalysis of sedimentary selenium toxicity data using only effects data for fish. (Van Derveer and Canton, 1997.)

mentioned his unpublished data indicating high sediment-to-benthos correlation in lotic (flowing-water) systems. Dr. Fairbrother said to measure total selenium and to consider lotic vs. lentic (standing water) differences. Dr. Adams said to measure total selenium, 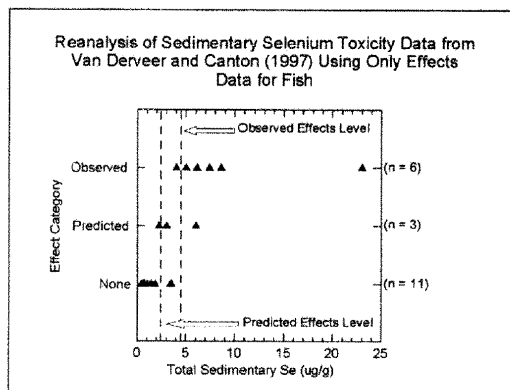because individual species have not been correlated with benthos. Dr. Fan said to measure proteinaceous selenium and seleno-methionine in benthos and detritus. Dr. Riedel said that better analytical methods are needed, and Dr. Skorupa said that a matched sediment and benthos study is needed.

Discussion:

Dr. Adams clarified that the lack of correlation between selenium species and benthos results from the lack of data on the subject. Dr. Fan said that her recommendation to measure proteinaceous selenium was based on an educated guess that detrital selenium is probably concentrated in peptides or proteins. Dr. Cutter agreed that this is a reasonable assumption. Dr. Fan added that her group performed an experiment in which they compared detrital material captured in a sediment trap to cored sediments. The material that settled in the trap (rich in detritus) contained an order of magnitude more selenium than did the cored sediments (Fan, unpublished).

Mr. Van Derveer presented his unpublished data from a study in the Middle Arkansas River Basin in Colorado (Figure 6). The graph was a log-log plot relating sedimentary selenium to selenium concentrations in chironomids. He pointed out that there seemed to be a positive relationship. The experts discussed the possibility of relating this information to the effects information in the previous graph to estimate a threshold of dietary selenium associated with effects in fish. Mr. Van Derveer agreed that this was a useful direction for research, but he stressed that far more data would be needed. Dr. Skorupa added that, to perform such an analysis, it would be important to know what the fish were actually eating. The experts discussed the possibility of using assimilation efficiencies and protein-normalized selenium values in food-chain modeling. The variety of food chains present in different habitats was also discussed; not only do lotic and lentic systems differ, but lotic systems have high- and low-energy areas.

Question 10: In priority order, which sediment quality characteristics (e.g., TOC, etc.) are most important in affecting the chronic toxicity and bioaccumulation of selenium to freshwater aquatic life under environmentally realistic conditions? Of these, which have been (or can be) quantitatively related to selenium chronic toxicity or bioaccumulation in aquatic organisms?

Discussion leader's summary of premeeting comments:

Mr. Van Derveer gave a brief summary of each individual's comments on this question. He said there was a reasonable level of agreement among those who responded. Everyone who responded
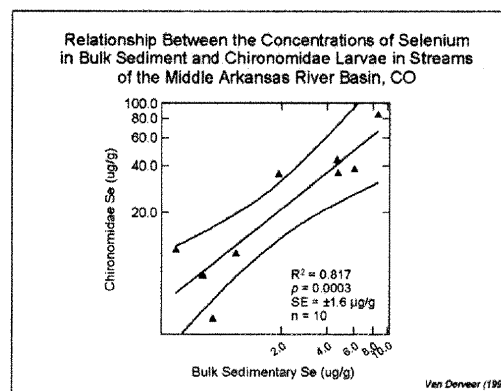

Figure 7. The relationship between the concentrations of selenium in bulk sediment and chironomidae larvae in streams of the Middle Arkansas River Basin, CO. (Van Derveer, unpublished.)

mentioned TOC may be important, although Mr. Van Derveer pointed out that they all cited the same

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systems, using 204 water-sediment pairs from 15 water bodies (Adams, unpublished). The correlation coefficient was 0.66

Correlating water with fraction of sediments coefficient of 0.68; with grained fraction the 0.73. Dr. Riedel pointed fish, temporal variability correlation; because temporally variable and well buffered, it is not the correlation is poor.

Mr. Van Derveer graph from his work to conversation (Figure 7). showed the product of selenium and TOC on the x-axis and selenium on the y-axis.

overall. the fine-grained yielded a the coarse-coefficient was out that, as with affects water is highly sediments are surprising that

showed another stimulate more This graph dissolved sedimentary sedimentary He noted that,

Figure 8. Western Streams Model. (Van Derveer and Canton, 1997.)

at least in streams of the western United States, there is a fairly predictable relationship. Dr. Cutter suggested revisiting the data with a normalization to aluminum in the low-TOC range (i.e., normalize to "TOC or aluminum"). Other experts said that it is important to consider whether systems are at equilibrium or not. (For example, is there an ongoing input?)
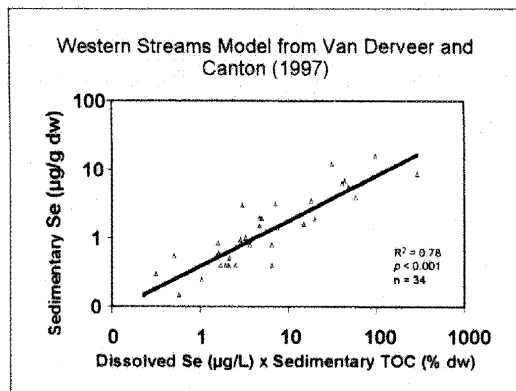
Research Needs

Dr. Fairbrother moved the conversation to the issue of research needs. Dr. Fan said there is a need to test the relationship among waterborne selenium, TOC, detrital selenium, total sediment selenium, and biota selenium for all abundant sediment species. Dr. Riedel said that it would be important to obtain the

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assimilation coefficients for different benthic organisms and to examine how the different types of selenium in the food affect these coefficients. Mr. Van Derveer said that the issue of whether or not organisms are depurated should be addressed. Dr. Cutter said that a coupled examination of the ecosystem and the biogeochemical cycle should be performed at a site. Mr. Van Derveer said that he would like to see a more mechanistic understanding of what affects selenium accumulation in the sediments. Dr. Skorupa said he would like to see more data linking the biology of the most sensitive species to the heterogeneity of the sediments; some species may feed preferentially in high-selenium areas (because of other characteristics of these areas). Dr. Fan agreed that she would like to see if selenium accumulation by benthos can be correlated with selenium levels in organic-rich sediments. Dr. Hamilton mentioned the issue of differential accumulation of selenium by closely related species (e.g., flannelmouth vs. razorback suckers). Mr. Van Derveer said that it would be useful to do some controlled laboratory studies using field-collected sediments, perhaps running EPA's *Lumbriculus* bioaccumulation test. Dr. Adams said he would like to see examination of the sites that have relatively high levels of selenium but no effects seen; he said that these sites should help shed light on mechanistic understanding of processes. Dr. Fan said it is important to understand the mechanism of toxicity; she cited a review article from the biomedical field (Spallholz, 1994), which she urged the other experts to read.

Wrap-Up

Mr. Van Derveer summarized the preceding discussion. After some further discussion, the experts agreed that the following was an accurate summary:

Elemental and organic selenium predominate in sediments. The process is somewhat redox driven, depending on the system type and the characteristics of the sediments. Selenium tends to be located in detritus. Total selenium may predict toxicity; there are some questions about relating selenium concentrations to TOC, the possibility of carbon-to-nitrogen (C:N) ratio normalization, normalization to proteins, and direct measurements of detritus vs. whole sediment. Spatial heterogeneity is an issue, as is preferential feeding (some species feeding in certain areas with high selenium concentrations). In addition, there are some issues with the power of biological assessments to detect effects. Concerning the question of what should be measured, there is some argument that total selenium in surficial sediments should be measured and it was also pointed out that multiple dietary pathways should be considered when they exist. Direct correlations of specific selenium forms to effects are lacking, but an overall causal relationship tends to exist, where high selenium in sediments tends to co-occur with effects at the population and community level. Some examples might be (1) effects seen in Belews Lake after the cessation of selenium input and (2) microbial community changes.

Which sediment characteristics appear to be most important? TOC seems to be important, but may be inappropriate for anoxic sediments where redox conditions are driving selenium accumulation; there may be some pseudocorrelation or a simple biogeochemical process moving selenium and sequestering it in sediment. Quantity of detritus may be important, and it may be important to measure that directly. In lentic systems, the residence time appears to be important; selenium accumulation can be calculated based on residence time and some other factors. Aluminum should be considered as a marker for inorganic sediment composition, to help differentiate detrital matter from inorganic material. Efflux from sediment to the water column is important. Sulfate may be important to sedimentary microbial communities, affecting selenium speciation. (Dr. Fairbrother noted that most items on this list reflect, not results reported in the literature, but things some or all of the experts think should be important, based on their understandings of the relevant processes.)

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Finally, relating sediment to water, a TOC model exists for western streams. Residence time is important for both lentic and lotic systems. Whether the system is at equilibrium or not should be considered. Uncertainty is moderate overall for relating sediment to water, based on the small number of publications specifically addressing this relationship.

Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.

Sediment is the dominant sink for selenium in aquatic ecosystems. Elemental and organic selenium tend to predominate in sediment, with elemental selenium dominating under reducing conditions. Organic selenium is believed to be markedly more bioavailable than elemental selenium. Sedimentary organic materials (detritus) are an important dietary resource for aquatic invertebrates. Selenium tends to accumulate in detritus, thereby entering the benthic-detrital food web.

The literature regarding the toxicological effects of sedimentary selenium is sparse, and most workshop participants relied upon two to three publications for preparing their premeeting comments. Several participants cited a paper by Van Derveer and Canton (1997), which concluded that the total sedimentary selenium concentration is a reliable predictor of chronic toxicity in fish and birds. A reanalysis of those data (Van Derveer, premeeting comments), focusing only on fish, indicated that toxic effects may occur when total sedimentary selenium concentrations exceed 4 μg/g (dry weight). The field data that were collected from Belews Lake after curtailment of fly ash input demonstrate the importance of sedimentary selenium in bioaccumulation and toxic effects on fish. Although waterborne selenium concentrations declined rapidly, Se concentrations in sediment and biota declined very slowly and teratogenic effects in fish populations persisted even 10 years later. Effects data for particular selenium forms in sediment are lacking in the literature; thus, preventing interpretation of sedimentary selenium speciation data.

The relationship between sedimentary selenium and toxicological effects may be affected by factors such as spatial heterogeneity in sedimentary selenium concentrations, habitat selection by different types of aquatic biota, and preferential feeding habits of aquatic biota. Moreover, efforts to relate toxicological effects to sedimentary selenium concentrations, or selenium concentrations in any environmental compartment, should consider the statistical power of the effects assessment. It was hypothesized that prediction of food web bioaccumulation and subsequent chronic effects on higher trophic levels might be improved by measuring detrital selenium, proteinaceous selenium in sediment, or seleno-methionine in sediment.

Unpublished data (Van Derveer, premeeting comments) were presented which indicate that a significant positive relationship exists between total selenium in surficial sediment (ca. 0-3 cm) and selenium accumulation in depurated Chironomidae larvae from streams of the middle Arkansas River basin, Colorado. These data suggest that, at least for some systems, total sedimentary selenium is well correlated with bioaccumulation in benthic organisms.

The following sediment quality characteristics were identified as potentially relevant to chronic selenium toxicity:

- Sedimentary TOC (possibly inappropriate for anoxic sediments where redox processes predominate);
- Quantity of sedimentary detritus present;
- Water residence time (longer residence time promotes greater sedimentary selenium accumulation);
- Normalization of sedimentary selenium to sedimentary carbon:nitrogen ratio;

- Normalization of sedimentary selenium to sedimentary protein content;
- Efflux of selenium from sediment to water; and
- Sulfate concentrations (may affect the composition of sedimentary microbial communities and thus the speciation of sedimentary selenium).

Sedimentary selenium can be related to waterborne selenium using two approaches, with a moderate degree of uncertainty. For streams of the western United States, a TOC-based model can be applied (Van Derveer and Canton, 1997). Sedimentary selenium accumulation in lentic and lotic systems can be calculated by considering residence time and applying a mass balance approach (Cutter, 1991). Because waterborne selenium concentrations tend to exhibit large temporal variations, the strength of the water-to-sediment correlation is affected by the averaging period selected. It is also important to consider whether the regime of waterborne selenium input to a system is relatively consistent over time (e.g., a stream receiving selenium from surrounding geological sources) or recently altered (e.g., Belews Lake after curtailment of fly ash input).

The following research issues were identified as being relevant to developing a more complete understanding of the role of sediment in chronic selenium toxicity:

- Assessing the relationship between detrital selenium and food web bioaccumulation;
- Understanding factors that may cause variability in selenium accumulation in benthic invertebrates, such as interspecific differences, assimilation rates, and effect of sedimentary selenium speciation;
- Evaluating the potential merit of depurating specimens prior to correlation with sediment, or any other environmental compartment;
- Correlating sedimentary selenium concentrations at preferred feeding sites with particular species of interest (e.g., endangered fish);
- Defining the mechanisms of selenium accumulation in sediment; and
- Performing laboratory studies of sedimentary selenium accumulation by invertebrates.

DISCUSSION SESSION 4:
Cross-Cutting Issues Associated With a Chronic Criterion

Dr. Fairbrother explained that the cross-cutting session was intended to capture issues that did not fit neatly in one compartment, as well as any other comments or ideas that any of the experts had not yet had a chance to raise. She listed the following issues to be discussed during the session: spatio-temporal variability and averaging times; ecosystem type (including lentic vs. lotic); site-specific approaches; analytical methods; sufficiency vs. toxicity; natural background; and interactions with other stressors.

Question 12: How does time variability in ambient concentrations affect the bioaccumulation of selenium in aquatic food webs and, in particular, how rapidly do residues in fish respond to increases and decreases in water concentrations?

Discussion leader's summary of premeeting comments:

Dr. Fairbrother summarized the experts' premeeting comments on this question as follows: Water concentrations can change by ten-fold in 1 month. Bioaccumulation in fish tissues changes over months. Phytoplankton and bacteria accumulate selenium rapidly (5-6 days), with turnover in 2 weeks. The rate-

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- Normalization of sedimentary selenium to sedimentary protein content;
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limiting step is the conversion of the inorganic form to the organic form. The $t_{1/2}$ for sediments depends on the form of selenium.

Discussion:

Dr. Cutter suggested that averaging time should be a function of retention time (the physics of the system), which varies greatly between lentic and lotic systems. Dr. Fan said that the biological component of a system can also have an effect on averaging time. Dr. Skorupa again raised the issue that a short-term spike can have long-term food-chain implications, based on the Maier et al. (1998) study. Dr. Fairbrother summarized that, in addition to the physics of the system, the biology of the system has to be considered, because organisms will have different effects on the residence time of selenium in the various compartments. Both physics and biology should be looked at when examining the relationship of water fluxes to responses or to fish tissue changes.

Question 13: To what extent would the type of ecosystem (e.g., lentic, lotic) affect the chronic toxicity of selenium?

Discussion leader's summary of premeeting comments:

Dr. Fairbrother summarized the experts' premeeting comments on this question as follows: There was general agreement that the type of ecosystem has a large effect on selenium cycling in the system. Lotic systems have a slower rate of conversion of inorganic to organic selenium, shorter retention time of carbon and decreased storage potential, and less accumulation of selenium in sediments. The modeling approach differs between lotic and lentic systems. Bacteria and phytoplankton species differ between the two ecosystem types, which may cause differences in bioaccumulation factors. Also, lentic systems have higher primary productivity. Open (rather than closed) fish populations make changes in recruitment more difficult to document.

Discussion:

Dr. Riedel added that lotic systems have a larger contribution of terrigenous detritus, which tends to dilute the selenium concentration. Dr. Fan replied that if the allochthonous input is through seleniferous soils, the reverse could be true. Dr. Skorupa said that another way in which lotic and lentic systems differ is that lotic systems are more likely to provide the source water for lentic rather than vice versa. Dr. Fairbrother replied that the reverse could also be true. Dr. Riedel said that the key point is not to consider parts of systems in isolation. Dr. Hamilton agreed that the interconnection of lentic and lotic systems is important. He cited a study by Radtke et al. (1988) on the Lower Colorado River, which showed that selenium in the backwaters was coming from the river's main stem. Conversely, a study by Engberg (currently in review) showed that only 18 percent of the selenium entering Lake Powell stays in the lake.

Dr. Adams said that there are other ecosystem types that should be considered, such as the Great Salt Lake, saline streams, ephemeral streams, and cold northern streams. He added that indigenous biology in each of the different environments should be taken into account.

Dr. Fairbrother questioned the statement that modeling approaches vary for different systems. She said that, in her opinion, the major components of the model are conceptually the same for different systems and that what varies are the rate processes. She asked for comments from the other experts. Dr. Fan replied

that components other than rates vary (e.g., food-web composition). Dr. Cutter replied that food-web composition is taken into account by Dr. Bowie's model. Dr. Bowie agreed.

Dr. Fan asked Dr. Bowie what was the minimum amount of information required to use his model for a site. Dr. Bowie said that one can use very little information and make guesses, but that the more actual data that are included, the better the model is. He said that the hydrology of the system and the selenium loadings would be the most important information, followed by the food web structure and some information on sediments. Dr. Fan replied that it is difficult to get a good mass balance for a dynamic system. She mentioned volatilization as an important component that is difficult to measure. Dr. Bowie replied that he didn't think volatilization was a major factor in most systems; further, the model takes into account factors which affect volatilization, such as the volatile fractions of bacterial and algal excretions. During the discussion, it was also clarified that the main purpose of the model is to be able to tie biological effects to water concentrations resulting from loadings, and possibly predict outcomes in hypothetical future situations.

Site-Specific Approaches:

Dr. Fairbrother summarized suggestions Dr. Adams made about different approaches for doing site-specific assessments. These were: (1) Empirical database of fish tissue concentration as a function of water concentrations (develop for a variety of species and couple with reproductive effect concentrations); (2) Apparent Effects Threshold (AET -- use it to identify areas where site-specific effects measurements should be done); and (3) Modeling approach (parameterize for the ecosystem of concern).

Discussion:

Dr. Adams elaborated further on the AET approach. He explained that it is the approach shown in the graph Mr. Van Derveer presented earlier (Figure 5). For multiple sites, concentrations of selenium in various compartments are coupled with information on the presence or absence of biological effects at the site. This approach identifies three ranges of concentrations: a range in which effects were never seen, one in which effects were sometimes seen, and one in which effects were always seen. This approach helps to establish rough effect thresholds and to identify sites for which more site-specific data are needed (i.e., those in the middle range). The AET approach has been articulated for marine sediments (Barrick et al., 1989). Dr. Bowie said that, for such an approach, using total selenium measurements might not be desirable for sediments, because detrital selenium is what gets into the food web. Dr. Fairbrother agreed that, in the sediments discussion session, there had been suggestions to normalize to TOC or protein. Dr. Fairbrother emphasized that, for the AET approach, it would be crucial to consider whether the studies used had adequate power to detect effects.

Dr. Fairbrother then asked Dr. Adams to discuss the idea of an empirical database. Dr. Adams said that this idea was based on various papers (e.g., Skorupa and Ohlendorf, 1991; Ohlendorf and Santolo, 1994). He said that, basically, this approach would again use information from multiple sites. Relationships between, for example, water concentrations and levels in fish reproductive tissue could be graphed and used to create a regression line. The strength of the regression's predictive power could be evaluated; in addition, as with the AET approach, sites with strong site-specific influences could be identified.

Dr. Riedel asked Dr. Adams how he would modify the water-to-fish regression if it did not fit well. Dr. Adams replied that his first step would be to remove sites like Belews Lake, in which there is not an

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ongoing selenium discharge. Dr. Skorupa said that it should not be too hard to separate out the sites causing the "noise" in the data, based on knowledge of site-specific factors. He expressed optimism that it would be possible to create a good global relationship between water-column and fish-tissue selenium. Dr. Cutter added that another factor to consider would be the amount each site is elevated above background for its region.

Dr. Fairbrother said that the experts seemed to be contradicting their conclusions from the previous day, in which most of them had said that water concentrations could not be used to predict fish tissue concentrations. Dr. Adams said that part of the reason for that conclusion was that, to date, efforts to build global models had not been very successful. Dr. Skorupa said that two different scales of analysis were being discussed. During the water session, the experts addressed the question of what confidence they would have in predicting fish-tissue selenium concentrations from water selenium concentrations. He said that that was a different question from the current issue, which was looking globally at relationships between water and fish and trying to identify sites that are over or under the regression line. Dr. Cutter agreed. Dr. Adams said that, even if tissue levels are considered to have the best predictive power of effects, they still must be related back to water concentrations, or the tissue-based approach leads only to site-specific assessments for every site. Dr. Fan added that picking apart the variables that make some sites deviate from the global relationship would lead to a better understanding of the relationship between tissue concentrations and water concentrations.

Dr. Fairbrother commented that what the two approaches under discussion would mainly show is which sites need site-specific studies. Dr. Riedel asked whether a "site-specific study" means anything beyond analyzing selenium in the discharge and the receiving body. Dr. Skorupa replied that, in his opinion, site-specific analysis usually boils down to developing rigorous effects data to assess whether effects are occurring at a particular site.

Analytical Methods:

Dr. Cutter presented the following remarks:

The Chemical Forms of Selenium in Natural Waters

DISSOLVED

Se(VI)	Selenate (SeO_4^{2-})
Se(IV)	Selenite ($HSeO_3^- + SeO_3^{2-}$)
Se(0)	Elemental selenium (insoluble, but may be colloidal and pass through a 0.4 μm filter)
Se(-II)	Selenide, primarily in the form of organic selenides such as seleno- amino acids (e.g., seleno-methionine, $CH_3Se(CH_2)_2CH(NH_3)CO_2H$) in dissolved peptides, and dimethyl selenide (($CH_3)_2Se$))

PARTICULATE

Se (IV+VI)	Adsorbed to mineral or biogenic phases
Se(VI)	Selenate esters in membranes
Se(0)	Elemental Se precipitated from water column or produced in sediments

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Se(0/-II)   Metal selenides (pyrite-like compounds)
Se(-II)   Organic selenides (primarily seleno- amino acids in proteins)

Factors to Consider for Selecting Appropriate Analytical Methods for Determining Selenium in Natural Waters

1. Accuracy. For obvious reasons, systematic errors must be eliminated. Standard additions method of calibration should be used and appropriate (i.e., same matrix type) standard reference materials should be analyzed (although only limited speciation data for these are available).

2. Precision. The analytical precision must be much less than the environmental variability in order to discern it.

3. Low detection limits. Natural concentrations of dissolved selenium can be as low as 2 ng Se/L, necessitating low detection limits. In this respect, for determining loadings, etc. a lack of data (i.e., below detection limits) should be avoided. Moreover, low detection limits allow potential interferences to be minimized via dilution. As a general rule, the detection limits should be approximately 10x lower than the expected concentrations.

4. Ability to determine dissolved _and_ particulate speciation. The speciation of selenium in both the dissolved and particulate phases has been shown to affect its bioavailability and/or toxicity.

Analytical Techniques for Selenium Determinations in Natural Waters

Method	Speciation		Interferences	Detection Limit	Relative Cost
	Dissolved	Particulate			
SHG AAS	yes	yes	few	2 pptr	$
SHG ICP-MS	yes	yes	few	<2 pptr	$$$$
Deriv.-fluorimetry	yes	no	many	5 pptr	$
Deriv.-GC	yes	no	few	5 pptr	$$
IC	yes	no	many	1 ppb	$
IC-ICP-MS	yes	no	many	<2 pptr	$$$$

SHG = selective hydride generation
AAS = atomic absorption spectrometry
ICP = inductively coupled plasma

What can we do now?

Dissolved: IV, IV + VI, total, selected or operationally defined organics
VI = (IV + VI) - IV
organic Se (-II) = Total - (IV + VI)

Particulate: IV, IV + VI, total, Se(0), pyrite-Se
organic Se (-II) = Total - (IV + VI) - Se(0) - pyrite-Se

Organic Se: The big problem. HPLC, etc. require knowledge about specific compounds. Can get at specific compounds or compound classes. For example: Copper-chelex gets primary amine Se; cation resin gets the selenonium cation.

Dr. Fan pointed out that the cost of disposal has to be factored into the cost of analysis using selective hydride generation, because a very acidic waste is generated for which disposal can be expensive. She added that her laboratory has had problems with their nebulizer becoming clogged. Dr. Cutter replied that a nebulizer is not necessary for his AA-hydride method.

Dr. Fan noted that selenonium can be analyzed for by spiking whole water with base and analyzing the resulting head space. She asked Dr. Cutter if he had tried using the copper chelex method to analyze for seleno-methionine in sediments, and he replied that he had not. Dr. Riedel said that his group, after dosing algae with selenium-75, had detected small amounts of free seleno-methionine in water (in the parts per trillion range) using copper chelex. Dr. Skorupa asked Dr. Cutter to comment on neutron activation. Dr. Cutter replied that this method does not do speciation and that special attention must be paid to sample preparation.

Dr. Cutter presented further remarks:

Water-Column Sampling

Sample
--> 0.4 um filter (immediate)
--> "dissolved" (pH <2 with HCl, borosilicate glass)
--> suspended particles (freeze; dry at low temp)

Why? Dissolved and particulate represent different "pools" available to different parts of food web.

Sediment Sampling

Box core (or equivalent)
--> "squeeze" and filter
--> dissolved
--> particulate (dry at low temp)

Why? Dissolved and particulate availability; fluxes; selenium changes with depth; preserve flocculent matter at surface.

References for sediment sampling: Bender et al., 1987; Blomqvist, 1985; Blomqvist, 1991; Jahnke, 1988; Zhang et al., 1998.

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For determination of selenium in sediments, Dr. Fan brought up benchtop x-ray fluorescence spectrometry. She said that it has the advantage of not requiring digestion, which minimizes sample handling and thus the potential for technician error. Dr. Cutter replied that the detection limits for this method are very high. Dr. Fan agreed, saying they are currently around 2 ppm, but she said the method could be useful for more highly contaminated sediments. She added that this technique determines other metals at the same time, which can be useful for looking at interactions. Dr. Cutter replied that it is an expensive instrument. Dr. Fan responded that it is not more expensive than other instruments he had referred to and that it results in large savings in labor costs.

Dr. Adams commented that Dr. Cutter's chart of analytical methods was a summary of the state of the art, rather than the methods commonly used. He said he thought a detection limit of 2 pptr was a stretch for some of the methods and was certainly a stretch for contract laboratories. Most contract laboratories, he added, are struggling to do a good quantitative analysis at the 2 ppb level. Dr. Riedel replied that EPA is currently publishing and validating a method for arsenic and that the selenium method will come in time. Dr. Cutter replied that, in his opinion, it is crucial that detection limits be ten times below the concentrations being analyzed. He added, however, that he understands the situation faced by a contract or utility lab analyzing large quantities of samples in short time periods. He said that, with EPRI funding, he had developed a methods "cookbook" currently used by many utility labs. He said that the approach he recommends for these labs is to analyze for total selenium, making sure that their method is accurate and precise, and to speciate a subset of samples.

Sufficiency vs. Toxicity:

Dr. Fairbrother introduced this topic by saying that selenium is a required micronutrient; the question, then, is whether the range between sufficiency and toxicity levels is large enough that we need not worry about sufficiency. Dr. Riedel responded that there are regions, such as places on the Canadian Shield, in which selenium concentrations are so low (in the low pptr in the water column) that algae respond to selenium administration. Dr. Fan added that she found that she needed to add selenium to an algal culture in her laboratory that she had isolated from an evaporation pond. Algal growth had been diminished, but was ameliorated when she added 10 ppb of selenium to the culture. Dr. Fairbrother pointed out that these algae were adapted to a high-selenium environment. She reiterated the question of how wide the zone between sufficiency and toxicity is, and Dr. Riedel replied that for plants and algae it is quite wide.

For fish, Dr. Hamilton cited a study in which a selenite-spiked diet was fed to rainbow trout (Hilton et al., 1980). The researchers determined that between 0.15 and 0.38 µg/g dry weight selenium in the diet was the sufficiency level; they estimated that the toxicity level was about 3 µg/g. Dr. Hamilton pointed out that this was only a ten-fold difference, which is fairly narrow. Mr. Van Derveer said that spiking with selenite did not realistically mirror an environmental exposure.

Dr. Cutter said that, in his opinion, one would not have to worry about making a system too clean. He pointed out that low-selenium environments would have an assemblage of species that were adapted to the lack of selenium. Dr. Skorupa agreed; he said that, in 10 years of research, he has never found selenium levels in a waterbird egg in the wild that were below the level of selenium sufficiency determined for chickens.

Dr. Adams said that published papers have established a selenium requirement for daphnids in the range of 0.5 to 1 µg/L added to the algal culture that is fed to the daphnids. He also commented that European

researchers have started to develop sufficiency-toxicity curves for metals and said that this is interesting because it allows one to look at the gradations of effect. He added that, in the Netherlands, water criteria for metals are adjusted for natural background concentrations. Dr. Fairbrother then turned the discussion to the topic of natural background.

Natural Background:

Dr. Fairbrother asked Dr. Cutter to elaborate on his assertion that 0.1 ppb is the natural background for selenium in U.S. freshwaters. He replied that the data he based this on were presented in a chapter he wrote on selenium in freshwater systems, which he had provided to the group (Cutter, 1989). He said that he only included data he considered to have been produced using sound analytical methods; he acknowledged that the western United States was not adequately represented. He also cited another reference he provided (Cutter and San Diego-McGlone, 1990), detailing variability in selenium concentrations over 2 years in the Sacramento and San Joaquim rivers. He added, however, that concentrations in the San Joaquim are affected by agricultural input, and that headwater data would be necessary to estimate natural background. Dr. Riedel said that using headwater data ignores the natural selenium inputs that occur as one moves downstream. Dr. Fan said that researchers had addressed this issue in the San Joaquim by looking at tracers; they determined that approximately 90% of the selenium inputs were agricultural. Dr. Fairbrother asked if this method could be used to determine natural background in systems with anthropogenic inputs. Dr. Fan replied that some researchers are trying to do this, but it is not yet a proven method. Dr. Adams questioned how one defines a number for "background," since there is a range of values; he cited some examples of water bodies with natural selenium levels much higher than 0.1 ppb.

Dr. Cutter turned the discussion to the natural background selenium level for U.S. freshwater sediments, which he said is about 1 ppm. Dr. Adams agreed. Dr. Cutter said there is not much regional variation. Dr. Skorupa said that the USGS study of surficial soils in the United States found little regional variation in selenium soil levels. Dr. Fairbrother questioned how numbers were averaged in this study, agreeing with Dr. Adams's comment that one must look at the distribution as well as the median. She summarized the discussion by saying that there is still debate about natural background and that more work must done to allow good determinations to be made of whether sites' selenium concentrations are at natural background or elevated.

Interactions with Other Stressors:

Dr. Fairbrother raised the issue of the interaction of selenium with other stressors, asking the experts whether they had confidence that effects seen in the empirical data set are due just to selenium. Dr. Cutter said that he did not have confidence that this was the case, because when there is an excess of selenium, there is often an excess of something else. Dr. Hamilton said that the literature is fairly limited on many other elements. He cited an example from his research; in a study he did on the Green River, vanadium was somewhat elevated and may have been a confounding factor, but he could only find one relevant study about vanadium. Dr. Fairbrother and other experts pointed out the additional problem of extrapolating from the laboratory to the field. Dr. Fan said that, as broad element scans are becoming easier to do, she is hopeful that more field data will soon be available. Dr. Skorupa said that he feels there are sufficient data establishing that effects attributed to selenium are actually caused by selenium alone. His group has done studies in reservoirs that have a suite of pollutants excluding selenium, and they have not seen the effects typically associated with selenium.

Clarification Requested by EPA:

At this point, Mr. Sappington asked the experts to clarify a couple of issues. First, he pointed out that, during the cross-cutting session, experts had discussed possible global approaches in relating tissue concentrations to water concentrations; however, during the water-column issues session the day before, experts had expressed skepticism about performing water-to-tissue correlations. He asked them to clarify this, and also to state some of the factors that they think might make the correlation poor. He asked whether the experts considered loading from sediments and spatio-temporal variability in the water column to be important factors.

Dr. Fan replied that the problem might be more complex than that and cited an example of an irrigation pond in California in which large changes in selenium load in bird eggs were observed with only a minor dilution of waterborne selenium concentrations, for unknown reasons. Dr. Fairbrother asked the experts to also clarify whether the form of selenium that is discharged to receiving waters changes the temporal or magnitudinal dynamics of what happens in the food chain. Dr. Cutter replied that it does; for example, the uptake rate of selenate is slow compared to selenite. Dr. Fairbrother said that part of the problem in trying to establish relationships is that the systems under study are generally non-equilibrium, dynamic systems.

Dr. Adams responded to Mr. Sappington's original question by agreeing that both mass in the sediments and spatio-temporal variability in the water column are important. He added that fish behavior is also important, including what fish feed on and where they forage.

Mr. Sappington asked whether the experts would expect tissue residue effect levels to differ between the laboratory and the field, or whether laboratory data are in fact useful for generating effect-level information. Dr. Hamilton replied that when he did laboratory studies, with both water-only and dietary exposure to selenium, he found the residue effect level to be very similar between the two; in other words, how the selenium got into the tissue did not affect the effect level. Dr. Riedel agreed that this is probably generally true, but that there are exceptions. He pointed out that there are many unknowns in the field, while organisms in the laboratory are kept under optimal conditions. Dr. Hamilton agreed.

Conclusions: The following summary of the entire discussion session was written by the discussion leader and reviewed by the other experts.

1. Spatio-temporal variability

There is a large amount of variability in selenium concentrations within compartments of an ecosystem (e.g., water, sediment, biota) across both time and space. The relationships between the compartments are not linear, however. Water concentrations may change rapidly (within days) whereas sediment concentrations take months or years to change, particularly in lentic systems. Fish tissue residues integrate all compartments and theoretically may change in response to alterations in any of them although food-chain exposures tend to dominate. Therefore, fish tissue residues also change over a period of months, and do not reflect the faster fluctuations of water.

The major factors influencing spatio-temporal variability are water residence time and biological processing (i.e., the type of organisms in the food web). The rate-limiting step may be the rate of conversion of

inorganic form to organic form, which is a function of the form of selenium and species of microorganisms in the sediment.

2. Ecosystem type

Ecosystems can be divided into lentic or lotic systems. Further subdivisions include ephemeral or perennial, highly saline, and northern (cold) streams. Differences in these systems that may lead to different responses to similar selenium input include retention time of carbon, rate of sediment accumulation, rates of conversion of inorganic to organic forms of selenium, and tolerance of local species. In addition, rates of allochthonous inputs (i.e., input of selenium materials from outside the aquatic system) versus autochthonous inputs (i.e., from within the system) differ. Most lotic systems are biologically open systems which makes it more difficult to measure ecologically-relevant effects on fish species that may move through the system, rather than being resident.

3. Site-specific approaches

Three approaches to site-specific assessments were proposed:

- Apparent effects threshold: This method would use existing field data to categorize systems as affected or not affected relative to selenium concentrations in sediment or water. The sediment/water concentration above which effects always occurred would be identified, as would the concentration below which effects never occurred. The concentrations in-between (where effects sometimes occurred or sometimes did not) would identify sites where a site-specific assessment would be needed.

- Fish tissue concentrations as a function of water concentrations: The empirical data from field studies that exist in the literature would be used to develop this bioaccumulation correlation on a global basis. Sites where measured fish tissue concentrations were different from the predicted concentrations, based on the amount of selenium in the water, would require a site-specific approach. If fish tissue – effects relationships are known for the species of concern, then sites could be further characterized as those with potentially higher than predicted effects or those with potentially lower effects.

- Modeling approach: The Aquatic Toxicity Model presented by George Bowie could be used to make *a priori* predictions of whether a concentration of selenium in water would result in effects to the fish. Site-specific input parameters include selenium input (amount, rate, and species), flow rates, water depth, and a few other hydrological parameters as well as food web species. The more site-specific data that are used in the model, the more likely is it to accurately predict effects.

4. Analytical methods

There are several methods for analyzing selenium in water, sediment, or tissue. No one method is the best for all media. Important considerations are desired minimum detection limits (ideally, should be ten-fold lower than the concentrations of interest), sample preparation requirements, and laboratory capabilities. Cost may be a factor as well. While methods are available that can achieve very low detection limits, many (if not most) contract laboratories are not set up to conduct these methods with appropriate accuracy or precision.

In addition to analytical methodology, appropriate sample collection and storage are required. Water samples should be acidified (with HCl) and kept cool; solid matrices should be kept frozen. Selenium may volatilize when a sample is heated and provide an incorrectly low value. Box core samplers are preferred

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for sediment sampling as they preserve the depth structure of the sediment, allowing measurements to be made on the upper flocculent (organic) material versus the lower inorganic portions.

5. *Sufficiency versus toxicity*

Since selenium is a required micronutrient for both plants and animals, there is an exposure concentration below which insufficiency effects are seen and a different concentration above which toxicity occurs. The area in-between is the Optimal Effects Concentration. For algae, there is a wide sufficiency zone and the required amount may differ depending on the amount of selenium in the system from which the test colony was derived (due to adaptation to a higher selenium environment). Fish have at least a ten-fold difference between required and toxic amounts. In general, there does not appear to be any naturally deficient systems, with the exception of some lakes in the Laurentian Shield area in Canada that may be deficient for algae. Furthermore, on a practical basis, it does not appear that source reduction of site remediation would result in systems with insufficient selenium concentrations. However, this issue may be important in laboratory studies where appropriate minimum concentrations of selenium must be provided to maintain colonies of test species.

6. *Natural background*

On the national level, the median background concentration of selenium in aquatic systems is about 0.1 μg/L. However, there is disagreement about this value and about the variability and range of natural background concentrations. Areas of highly seleniferous soils in the western U.S. may have naturally higher background concentrations either through movement of soils into waterbodies or into groundwater. Methods are being developed for differentiating between natural and anthropogenic inputs of selenium into an aquatic system, but there remains a great deal of uncertainty in the follow-on calculation of what a resulting natural background concentration would be.

7. *Interactions with other stressors*

Selenium has the potential to interact with other metals, causing either greater or lesser responses than predicted from selenium alone. Furthermore, exposure to selenium may reduce an organisms' ability to respond to other environmental stresses, such as has been shown for fish similar to those found in Belews Lake that were exposed to cold temperatures during laboratory studies (Lemly, 1993c, 1996). These types of interactions might confound the global empirical dataset relating effects to selenium concentrations in water, sediment, or food. Examples where this may have occurred include interactions between vanadium and selenium in a field study of fish reproduction. On the other hand, another study showed that effects were correlated only with the selenium concentration in the food, and that additional elements had no discernible effects. The endpoint of interest also may affect the potential for interactive effects to occur.

IV. OBSERVER COMMENTS

At the end of each day of the meeting, Dr. Fairbrother opened the floor to comments from observers. These comments are summarized below. In addition, observer presentation materials may be found in Appendix F.

Peter Chapman, EVS Consultants

This observer (speaking on the first day of the meeting) noted that discussions to date had mostly focused on standing-water systems. In contrast, his interest is flowing cold-water streams, particularly in Alaska and southeast British Columbia, with inputs of selenium from hard-rock mining and coal mining. He pointed out that these systems are quite different in many aspects from the systems under discussion by the experts. To date, his group's studies have found no adverse effects in streams in British Columbia with concentrations of selenium as high as 65 μg/L. He urged the experts and EPA to consider three key points:

- Flowing-water systems are very different from standing-water systems; much higher concentrations can be tolerated without adverse effects.
- Site-specific factors are incredibly important.
- Not all waters or biota require the same level of protection.

Philip Dorn, Shell Development Company

This observer questioned the need for a revision of the national freshwater chronic water quality criterion for selenium. He argued that no compelling field effects have been demonstrated in waters with selenium levels below the existing 5 μg/L chronic criterion. In addition, analytical methods for compliance testing are limited below 10 μg/L. Finally, there is large uncertainty in making correlations at the national scale between water-column selenium concentrations, selenium concentrations in the food chain, and selenium concentrations in bird eggs. He urged EPA to move toward developing site-specific residue- or effects-based criteria. He also noted that the cost per pound to remove selenium from discharge is quite high and that the removal process generates a large volume of sludge which must be disposed of. He asked EPA to ensure that future regulations are developed upon fact-based science.

Rob Reash, American Electric Power

This observer made comments on behalf of the Utility Water Act Group (UWAG), an association of electric utility companies and trade associations. UWAG is interested in EPA's reevaluation of the freshwater chronic aquatic life criterion for selenium because selenium is a natural trace element in coal and

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many of UWAG's members use coal as the primary fuel for electrical generation. The observer said that UWAG views a universal numeric chronic criterion for selenium as inappropriate. He urged EPA to consider the following issues:

- Stratification by waterbody type;
- Accurate accounting of site-specific factors affecting selenium toxicity; and
- Development of site-specific criteria technical guidance.

In addition, he offered the opinion that fish liver is a good tissue in which to measure residues if ovaries are unavailable; in his work, he has found that fish liver tissue mirrors water-column selenium concentrations.

Walter Kuit, Cominco, Ltd.

Speaking on behalf of Cominco Alaska, this observer said that selenium is a key issue at his company's Red Dog Mine in northern Alaska. An impending NPDES permit will lower the mine's selenium discharge limit to a level that the company cannot meet. He said that flowing streams should be considered separately from standing water and urged EPA to move quickly in developing site-specific guidance. He also asked EPA to provide preliminary guidance on possible changes in sampling procedures (e.g., implementation of fish ovary sampling), so that affected parties can start gathering relevant data as soon as possible.

William Wright, Montgomery Watson

This observer, an ecologist, is managing the Southeast Idaho Phosphate Resource Area Selenium Project. This project involves the evaluation of a 1,200-square-mile area containing 14 mines, where selenium is leaching from interburden waste shales. Receiving waters are typically intermittent tributaries of montane trout streams and are generally sulfate rich. Sampling to date has found water-column concentrations of selenium ranging from below detection limits to 2,000 ppb. Ninety percent of the selenium is in the selenate form. His group does not have definitive results yet, but has seen no adverse effects so far. Healthy populations have been found in areas with high concentrations of selenium. He echoed Peter Chapman's comments, saying that site-specificity is important, and beneficial use should be taken into account.

Chris Stanford, JD Consulting

This observer expressed the opinion that we have a long way to go in regard to quantifying the behavior and effects of selenium in the environment. He added that although revising the chronic criterion is a good goal, we do not yet have enough information to be able to develop a new nationwide criterion that is a definite improvement over the existing one. The solution to this in the short term, he said, is to develop site-specific standards, including guidance on sampling and data analysis and interpretations. In addition, he asked EPA to establish standards that can serve as guidance to contract laboratories.

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John Goodrich-Mahoney, EPRI Environment Division

This observer said that EPRI will be coming out with their Selenium Aquatic Toxicity Model this fall. He invited experts and observers to be beta testers for the model. He can be contacted at <jmahoney@epri.com>. He added that EPRI encourages EPA to develop site-specific guidance and is willing to offer any assistance it can.

Judith Schofield, DynCorp

This observer stated that DynCorp has been providing support to EPA in the development of 1600-series analytical methods; she updated the attendees on the status of the two methods that apply to selenium. EPA Draft Method 1638 is an ICP-MS method with an estimated detection limit of 0.45 µg/L. EPA Draft Method 1639 is a gas furnace-AA method with an estimated detection limit of 0.3 µg/L. The methods and their detection limits will be tested in upcoming interlaboratory validation studies. Formal proposal of the methods will probably occur in early 1999. She added that EPA is also working on a streamlining rule, which is a performance-based measurement system approach to analytical methods.

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Santa Clara Valley Audubon Society
Founded 1926

January 6, 2004

Mr. John Forren
U.S. Environmental Protection Agency
1650 Arch Street, Philadelphia, PA 19103

REC'D JAN 0 9 2004

Re: Impose restrictions of mountaintop removal mining to limit environmental damage

Dear Mr. Forren,

On behalf of the nearly 4000 members of the Santa Clara Valley Audubon Society and myself, I wish to voice our alarm and concern at the Bush administration's plan to continue allowing coal production to destroy the Appalachia region with mining practices that level mountaintops, wipe out forests, bury streams, and destroy communities. Many of our members travel to the region most impacted by these practices to enjoy its natural resource and wildlife values. In doing so, they support the local economies in a relatively sustainable manner. Thus, while seemingly far from the damage, our members have seen the devastation for themselves. This devastation will be further aggravated by the continuation of these poor resource extraction policies.

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According to the administration's draft Environmental Impact Statement (EIS) on mountaintop removal coal mining, the environmental effects of mountaintop removal are destructive, large-scale and permanent. Nonetheless, the draft EIS proposes elimination of mitigation measures such as placing restrictions on the size of valley fills that bury streams or limits on the number of acres of forest that can be destroyed through these practices. The administration offers little or no protections for imperiled wildlife and few safeguards for the communities that depend on the region's natural resources.

It is simply outrageous that the administration's "preferred alternative" for addressing the problems of the environmentally devastating effects of mountaintop removal mining would weaken existing environmental protections. The proposal is worse than current practices, which have been proved so harmful. This "preferred alternative" ignores the administration's own studies detailing the devastation caused by mountaintop removal coal mining, including:

1-10

- Over 1200 miles of streams damaged or destroyed by mountaintop removal to date;

- Forest losses in West Virginia with the potential of directly impacting as many as 244 vertebrate wildlife species;

- An additional proposal of 350 square miles of mountains, streams, and forests to be wiped out by mountaintop removal mining.

My fellow members of the Santa Clara Valley Audubon Society and I strongly urge you to consider alternatives that reduce the environmental impacts of mountaintop removal. Thank you for giving us an opportunity to voice our views on this important issue.

Sincerely,

Craig Breon
Executive Director

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January 6, 2004

Mr. John Forren
U.S. EPA (3EA30)
1650 Jacob Street
Philadelphia, PA 19103
mountaintop.r3@epa.gov

RE: Ohio Coal Association Comments on the Mountaintop Mining/Valley Fill
     Draft Environmental Impact Statement

Dear Mr. Forren:

The Ohio Coal Association joined with the National Mining Association (NMA) and
other state coal associations from Kentucky, West Virginia and Virginia in the delivery of
joint comments on the Draft Programmatic Environmental Impact Statement (PEIS)
addressing mountaintop mining and valley fills (MTM/VF) in the steep slope
Appalachian coal fields. The Ohio Coal Association fully supports those comments.

The Ohio Coal Association is a non-profit trade association that is dedicated to
representing Ohio's underground and surface coal mining production. The Association
represents close to forty coal producing companies and over fifty Associate Members,
which include suppliers and consultants to the mining industry, coal sales agents and
brokers and allied industries. As a united front, the Ohio Coal Association is committed
to advancing the development and utilization of Ohio coal as an abundant, economic and
environmentally sound energy source.

A common thread among the state industry groups joining in the above noted comments
is the fact that all conduct coal mining operations within the Huntington District of the
Corps of Engineers.

However, there are also some major differences between coal operations within the PEIS
study area and coal operations in the State of Ohio. In addition to the joint comments
filed by the National Mining Association on behalf of the Ohio Coal Association the
Association wishes to address the following specific concerns regarding the PEIS:

- **Applicability of PEIS to mining activities not involving MTM/VF outside of the
  study area**
  The Study Area established for the PEIS was based upon where MTM/VF activities were
  located in the past and where MTM/VF activities were anticipated in the future. Ohio
  was not included in the Study Area, and impacts of Ohio coal mining activities were not
  specifically studied as part of the PEIS. One exception however was a single study on
  the recovery of reclaimed streams in central Ohio, which was included as supplemental

material. As noted, this study did not involve valley fills. The research was conducted
years ago by the Office of Surface Mining and provided positive results.

There was an attempt in the document to outline assumptions that would provide some
correlation of MTM/VF activities in the study area to other mining activities in other
areas, but these explanations fell short of acceptable. No justification can be found for
expanding findings beyond the study area, or for adequately addressing impacts other
than those associated with mountaintop mining and associated valley fills. The document
should be modified to clarify that findings and recommended alternatives are not to apply
to mining activities outside of the study area that do not involve valley fills.

1-13

- **Authority for the Corps' new "no net loss of stream function" policy**
  There is no explanation and no justifiable authority found for the recent shift in Corps'
  policy to require no net loss of stream length and function, and yet the contents of this
  PEIS seem to be based almost entirely on this policy. There is even a statement in the
  document that claims that the goals of the CWA cannot be accomplished unless stream
  function is addressed (page 1-4). The document should be expanded to clarify this
  statement.

Everyone is aware of the no net loss of wetland policy that was officially expanded to
include no net loss of wetland functions. However, recent activities within the Corps
have now resulted in a no net loss of stream function and there is no clear indication as to
how this became official national policy. The Ohio Coal Association can find no official
document mandating the use of this policy. Only that it is now policy.

5-7-1

While wetland functions are easily identifiable and understood, this is not the case for
streams. In addition, the use of biological protocols to assess the range of stream
functions is inappropriate, especially in the case of ephemeral streams and the upper
reaches of intermittent streams. The US EPA went through an educational process on
wetland functions and provided opportunities for public input prior to implementing the
policy change from no net loss of wetlands to no net loss of wetland functions. This was
not the case for the stream policy now being imposed by the Corps.

- **Use of a headwaters category**
  The use of a "headwaters" category artificially increases the value of the majority of
  streams included in that category, namely 1st, 2nd and 3rd order streams, or ephemeral
  streams and upper reaches of intermittent streams. Through the use of the headwaters
  category an ephemeral stream will have the same value as perennial streams within the
  watershed because all would be considered as headwater streams. This then exaggerates
  the mitigation requirements to be imposed by the regulatory agency. The PEIS should
  retain the descriptions of ephemeral, intermittent and perennial for stream categorization.

5-7-4

- **Watershed approach to mitigation**
  The Corps is proposing to consider watershed needs when imposing mitigation
  requirements. The Ohio Coal Association agrees with this approach. However, the
  Corps should also determine impacts of a proposed activity on a watershed basis and not

5-6-3

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on a stream by stream basis. As an example, impacts to an individual ephemeral stream will appear significant when considering only the impacts to that individual stream. However, when you consider the impacts to that ephemeral stream relative to the watershed and downstream functions, the temporary loss of that ephemeral stream will be minimal at most. The Corps should make the necessary changes to reflect this more reasonable approach.

5-6-3

The Ohio Coal Association appreciates the opportunity to become involved in this process.

Sincerely,

Michael T.W. Carey
President

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RECʹD JAN 0 5 2004

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ASSOCIATE MEMBERS

GOV. BILL RICHARDSON
New Mexico

GOV. GEORGE PATAKI
New York

GOV. JOHN H. HOEVEN
North Dakota

EXECUTIVE DIRECTOR

GREGORY E. CONRAD

January 2, 2004

John Forren
U.S. Environmental Protection Agency
3ES30
1650 Arch Street
Philadelphia, PA 19103

Dear Mr. Forren:

This letter constitutes the comments of the Interstate Mining Compact Commission (IMCC) regarding the draft programmatic environmental impact statement on mountaintop mining/valley fills in Appalachia. IMCC is a multi-state governmental organization representing 20 mineral-producing states throughout the U.S., 15 of which operate federally approved regulatory programs pursuant to the Surface Mining Control and Reclamation Act of 1977 and most of which operate state programs/plans pursuant to the Clean Water Act. IMCC has participated at various times throughout the development of the draft EIS and in the preparation and review of the various technical studies that accompany and serve as the basis for the EIS. However, for the most part, IMCC has relied upon the expertise and input of the three primary states that have been the focus of the draft EIS, i.e. West Virginia, Kentucky and Virginia. In this regard, IMCC endorses the comments of the Commonwealth of Virginia that have been submitted on the draft EIS.

One of our primary concerns from the outset has been the development and identification of the appropriate alternatives that frame the basis of the draft EIS. Although the authors have come closer to the mark in the final draft, we still believe that the "no action" alternative (which is our preferred alternative) does not accurately reflect the realities of today's regulatory programs. In this regard, we echo the comments of Virginia that the no action alternative should be recharacterized as an option that would continue the existing SMCRA, EPA and Corps of Engineers regulatory programs, including past and ongoing amendments to those programs. We have seen a plethora of changes over the past several months in all three regulatory programs, many of which are being considered for adoption by the states, that reflect the ever-changing regulatory landscape associated with mountaintop mining and valley fills. It is essential that all three federal agencies continue to work cooperatively together, along with the states, to insure the implementation of comprehensive, realistic and legally sound regulatory programs that effectively protect the environment while maintaining and assuring an adequate supply of coal, our Nation's most abundant energy resource.

1-1

1-13

We are also concerned that the draft EIS, and its various recommendations, will have impacts and repercussions far beyond Appalachia. IMCC has articulated this view from the outset and our review of the draft EIS has heightened our concern. While EPA, OSM and the Corps have repeatedly stated that the EIS is focused only on Appalachia, it is difficult for us to believe that the alternatives being considered would not result in national rules, policies and guidelines that would impact other states' regulatory programs. In many instances these proposed changes would be either inapplicable or meaningless, due to the differences in geology, climate and terrain among the states. We urge all three federal agencies to be mindful of the "spill over" effect from the draft EIS and to guard against unnecessary and inappropriate impacts and intrusions to state programs.

1-13

Finally, should the federal agencies choose to move forward with the EIS (a course of action we do not support), we urge them to be mindful of the fact that in almost every instance, the states have the lead in implementing the applicable regulatory programs and thus any recommendations for action (in the way of regulations, guidelines and/or policies) should seriously consider the potential impacts on existing state regulatory programs and the implementation thereof by the states, especially in the context of permitting and enforcement.

Should you have any questions or require additional information, please do not hesitate to contact us.

Sincerely,

Gregory E. Conrad
Executive Director

Comments Regarding The Draft Programmatic Environmental Impact
Statement
July 24, 2004

Kent DesRocher
President and General Manager
Arch Of West Virginia

On Behalf Of The
West Virginia Coal Association

My name is Kent DesRocher and I am President and General Manager of Arch of West Virginia located at Yolyn, West Virginia. I have worked in the mining industry for nineteen years and in Central Appalachia for 10 years.

Over the past several years, coal companies have began to help diversify the economy of the fourteen coalfield counties. Through the development of post mine land sites including such diverse projects as industrial parks; golf courses; race track; recreational areas; commercial fish facility; housing; and public facilities, additional jobs are being provided for our children.

11-9-5

With the assistance of the West Virginia Coal Field Development Office, we are now even more capable to plan for the diversification of the economy in the coalfields. All fourteen counties have suffered from the

lack of transportation and developable acreage for many years. The transportation routes are improving with the upgrading of US 119 (Corridor G) and Interstate 77 (West Virginia Turnpike) coupled with Interstate 64 and 79. The development of the King Coal Highway and the Coal Fields Expressway will further increase development opportunities.

The mountainous terrain of the fourteen counties has also slowed growth in the area. Industrial, commercial and housing sites have been at a premium. The development of flat to gently rolling sites will assist in the growth and stability of the area.

Charles Yuill of West Virginia University lists six provisions for new land uses and land use opportunities.

1. Mr. Yuill indicates "most potential future mountaintop mining areas will be reclaimed to various forest cover". The current rules relating to commercial forestry must be reviewed to allow for the highest yield practical. The rules must be reviewed with respect to compaction; competition, and composition of soils. Recent studies would indicate that the best method has not yet been proposed to provide the best opportunities for commercial forestry.

10-3-5

2. Much discussion has occurred over the past several years regarding the post mine land use for agriculture such as vineyards, animal production; green house farming and aquaculture. Most of the sites where agriculture has been proposed will not occupy the entire site and approval of multiple uses will be required. For example, let's say the primary post mine land use is a vineyard, which would occupy fifty percent of the property. But since this is an agricultural project which is a higher and better use, the remaining portion of the property must be allowed to be developed into support areas, pasture lands or habitat which would not compete with primary higher use. Rules development must keep these issues in mind.

3. The study projects that "significant acreages of land suitable for developed post-mining land uses will result from future mining under all of the mining scenarios." The only way that the fourteen counties can significantly change the economy of the area is the development of large sites capable of supporting multiple uses. Mining scenarios that produce many acres of flat to gently rolling land areas can provide the opportunity to diversify and improve the economy of southern West Virginia.

10-3-5

4. Mr. Yuill is correct when he states that "Development limitations such as poor accessibility and infrastructure proximity will continue in nearly all of these areas." These issues will require the development agencies and environmental agencies to think out of the box. Such issues as the use of mitigation payments for water and sewer projects should be considered if there is a desire by the involved parties to redevelop and diversify the area. Smaller sites, less than 50 acres, will do little to diversify the economy of the 14 counties.

5. The environmental regulatory agencies must work closely with planning and development agencies when considering post mine land use. Here again, in order to allow for diversity and stabilization of the economy, regulatory agencies must think outside the box. Higher and better use must be site specific based upon many items normally associated in planning documents.

6. If we want the fourteen counties discussed in the study to diversify their economy, they must be allowed to create lands suitable for development. The sites must be of sufficient size to allow to make it worthwhile to provide the necessary infrastructure required for development.

10-3-5

With the advent of a responsible Environmental Impact Statement and a desire by the federal and state regulatory agencies to provide for affordable energy while providing sites for future economic transformation in the fourteen counties, we can provide a positive outcome for the citizens of the area.

In summary, large-scale surface mining can help support the development of infrastructure, access, and sites necessary for future development to allow for diversification of the economy in southern West Virginia.

Thank you for your time today.

Kent R. DesRocher

281 Ridgeview Terrace

Chapmanville, WV 25508

7/22/03

10-3-5

Partners in Flight
Northeast Working Group

John Forren
U.S. EPA (3EA30)
650 Arch Street
Philadelphia, PA 19103

Dear Mr. Forren:

Please accept the following comments in review of the Draft EIS on mountaintop coal mining and associated valley fills in West Virginia, Kentucky, Tennessee, and Virginia. These comments reflect discussions among members of the Northeast Working Group of Partners in Flight (PIF) regarding the likely impacts of mountaintop mining activities on the full suite of priority birds associated with mature deciduous forests, including populations of Cerulean Warblers, as well as a summary of landbird conservation priorities for the geographic area under consideration for the DEIS. A brief summary statement is presented below, with a more detailed discussion in the attached pages. These comments represent a synthesis of information gained from published literature, bird conservation plans developed by PIF, an extensive Cerulean Warbler Atlas Project conducted from 1997-2000, and discussions with colleagues. Figures from the Draft EIS on cumulative impacts of this mining activity in the study area suggest a massive and permanent impact within the EIS study area on the entire suite of priority mature forest birds (e.g., Cerulean Warbler, Louisiana Waterthrush, Worm-eating Warbler, Kentucky Warbler, Wood Thrush, Yellow-throated Vireo, Acadian Flycatcher) due to the estimated forest loss of approximately 760,000 acres from issued and future permits during the 20-year period of 1992 to 2012. Total cumulative forest loss from all mining activities, including permitted activities prior to 1992, is estimated at 11.5% of the total forest cover in the EIS study area. We consider this level of habitat loss to constitute a significant negative impact for the entire mature forest suite of birds, and especially for the Cerulean Warbler, the forest species of highest concern in this area. The cumulative impacts from issued and proposed future mountaintop mine/valley fill permits during this period appear likely to eliminate breeding habitat for 10%-20% (our estimate is 17%) of the global population of Cerulean Warblers. This level of habitat loss is unacceptable for a species that has experienced steep population declines over the last 30 years and is facing other major threats. Furthermore, research within the EIS study area shows that densities of Cerulean Warblers are reduced in isolated forest patches left by mining and near mine edges, indicating an even greater impact beyond the direct habitat loss from mining activities. According to PIF bird conservation plans, mature forest birds are a high conservation priority within the EIS study area, whereas grassland birds are not. In addition, the creation of poor quality, early-successional habitats that may be suitable for some shrub nesting species does not justify, or in any way compensate, the removal and fragmentation of extensive mature forest areas within the EIS study area. We encourage every effort to minimize the removal and fragmentation of existing mature forest habitat in the EIS study area.

Sincerely,
Randy Dettmers, Chair
Northeast Working Group of Partners in Flight
300 Westgate Center Drive
Hadley, MA 01035

8-1-2

9-1-2

7-3-3

Impacts of Mining Activities on Mature Forest Birds. The mountaintop removal mining/valley filling practices addressed by the EIS occur throughout what can be considered the core of the breeding range for many of the PIF high priority birds of eastern mature deciduous forests, including Cerulean Warbler, Louisiana Waterthrush, Worm-eating Warbler, Wood Thrush, Yellow-throated Vireo, and Acadian Flycatcher. According to Breeding Bird Survey (BBS) data, all of the species just mentioned occur at or near their peak abundances within the EIS study area, which largely overlaps with the Northern Cumberland Plateau physiographic area as delineated by PIF. Numerous other species of this habitat suite also occur in high relative abundances within this area, including Kentucky Warbler, Eastern Wood-Pewee, Ovenbird, and Scarlet Tanager. The mining and valley fill activities addressed by the EIS directly affect several of the primary habitats used by these species -- mature deciduous forest on Appalachian ridge tops (used by Cerulean Warbler, Yellow-throated Warbler, Eastern Wood-Pewee, Scarlet Tanager, Ovenbird, Wood Thrush), and mature mixed-mesophytic forest along headwater streams ("coves" -- used by Cerulean Warblers, Louisiana Waterthrush, Worm-eating Warbler, Kentucky Warbler, Acadian Flycatcher, Wood Thrush). Preliminary figures from the EIS on cumulative impacts of mining activities in the study area suggest a massive and permanent impact on the mature forest suite of birds within the study are due to the estimated forest loss of approximately 760,000 acres from issued and future permits during the 20-year period of 1992 to 2012. An additional 648,000 forested acres appears to have been lost from permitted mining activities prior to 1992.

9-1-2

The total cumulative forest loss from mining activities equates to an 11.5% reduction in total forest cover in the study area. Removing >10% of the forest cover from a region is likely to have negative impacts on mature forest birds, even in well-forested landscapes. As overall forest cover drops in a region, negative impacts to forest breeding birds from fragmentation and edge effects will become more severe. Work by O'Connell et al. (2000) across the Mid-Atlantic Highlands region, which includes a large part of the EIS study area, suggests that as landscapes fall below a threshold of about 82% forest cover, the ecological integrity of the forest community becomes increasingly compromised. Removing almost 12% of the forest from the EIS study area through mining activities alone will bring the % forest cover of this entire area down close to this threshold and certainly will cause some landscape-level areas within this larger area to fall well below this threshold. We consider the level of breeding habitat loss resulting from permitted and proposed mining activities to represent a significant negative impact for the suite of mature deciduous forest birds in the EIS study area, particularly for those species for which this area represents the core of their breeding range.

Specific Impacts to Cerulean Warblers. Because the Cerulean Warbler is the mature forest species of highest concern according to PIF assessments and because it has been petitioned for listing under the Endangered Species Act, we provide a more detailed analysis on the impacts that mining activities are likely to have on this species.

8-1-2

Population status and trends. The general status and population trends of Cerulean Warbler in most parts of its range are fairly well documented. These have been previously summarized in the USFWS Status Assessment (Hamel 2000), as well as final report to USFWS of the Cerulean Warbler Atlas Project (Rosenberg et. al., 2000). We believe that population trends as reported by the BBS are sufficiently reliable for Cerulean Warbler at range-wide and regional scales. These trends show a roughly 4.5%-per-year decline range-wide since1966, with steep declines in nearly

every region including in the core of the species' range, which overlaps almost entirely with the EIS study area.

As part of the development of a PIF North American Landbird Conservation Plan, estimates of the total continental breeding populations of most species have been developed for the purpose of setting conservation objectives. Using this method of extrapolating BBS relative abundances, the current total population estimate (using data from the decade of the 1990s) for Cerulean Warblers is about 560,000 birds, or roughly 280,000 pairs. Based on the BBS data, an estimated 70% of the total breeding population occurs in the Ohio Hills and Northern Cumberland Plateau physiographic areas, from southern Ohio and Pennsylvania, through West Virginia to Tennessee. Vast areas of suitable habitat in this region support large populations of Cerulean Warblers, especially on privately owned forestlands. We should note that although 280,000 pairs seem like a sizable population, it is among the smallest populations of any passerine bird in North America, which mostly number in the millions.

Threats to populations. We consider the major threats to Cerulean Warblers to fall within four main categories: (1) direct loss of breeding habitat from mining activities; (2) loss of breeding and migration stop-over habitat due to development; (3) loss of suitable breeding habitat from silvicultural practices; and (4) habitat loss on wintering grounds in South America. We consider the practice of mountaintop removal mining/valley filling to be the greatest immediate threat within the core of the Cerulean Warbler's breeding range.

Applying similar methods to those used in calculating total population sizes for the PIF North American Landbird Conservation Plan, BBS survey data indicate that the average breeding density of Cerulean Warblers across the Northern Cumberland Plateau physiographic area during the 1990s was 0.065 pairs/acre. Most of the EIS study area occurs in this physiographic area. This estimate does not include a time-of-day correction used in calculating the total population size, and therefore might be an underestimate. However, this density is similar to breeding densities estimated from territory mapping plots surveyed in southern West Virginia, although locally higher densities were observed in some locations. Using this BBS-derived estimate of breeding densities and applying it to the estimated forest loss of approximately 760,000 acres from issued and future mining permits between 1992 and 2012, habitat for approximately 49,400 pairs (17% of the estimated total Cerulean Warbler population) would be eliminated through mining activities during this period. This is a very rough estimate of the number of birds likely to be impacted and is based on the assumption that the entire area within permit boundaries would be disturbed. Nonetheless, we are confident in stating that breeding habitat for as much as 10%-20% of the known Cerulean Warbler population is likely to be directly eliminated by proposed and permitted mountaintop mines/valley fills during the 20-year period of 1992-2012. These numbers reflect direct loss of breeding habitat and do not reflect reductions in habitat suitability around mine sites. Research within the EIS study area has shown that densities of Cerulean Warblers are reduced in forest patches remaining from mining activities and in forest near mine edges. We consider the level of breeding habitat loss due to mining activities in the EIS study area to represent a significant negative impact for this species of high continental concern that is already experiencing steep population declines and is threatened by other major impacts such as development and loss of wintering ground habitat.

Relative Conservation Value of Reclaimed Mines vs. Undisturbed Forest Habitat. We do not consider removal of extensive areas of mature forest and replacement with the poor quality, early-successional habitats resulting from current reclamation practices to be an appropriate

8-1-2

action for bird conservation in the EIS study area. First, this habitat alteration is occurring in core breeding areas for many high priority birds of the mature eastern deciduous forest suite. Removing almost 12% of the forest cover from this area is likely to negatively impact all of these species. In particular, this area is critical for the long-term persistence of the Cerulean Warbler and the estimated forest loss from mining activities will represent a significant negative impact for this species of high continental concern. Second, current reclamation practices result in large acreages of grassland habitat, but the grassland suite of birds is a relatively low PIF conservation priority in the EIS study area. The vast majority of grassland bird species benefiting from the current mining activities are rather low in conservation priority, and this area is not a core breeding area for grassland birds. Third, current methods of reclamation following mountaintop removal mining/valley fill activities result in poor quality, early-successional habitats of grasses and shrubs that are likely to remain in these early-successional conditions for very long periods of time due to the soil disruption and compaction during the mining and reclamation process. Estimates of the length of time it will take tree species to colonize and re-forest these areas are in the many hundreds of years (e.g., 500-1000 years). The minimal value that habitats reclaimed under current methods might have for early-successional bird species does not justify replacing mature forests with extremely long-lasting, poor-quality, early-successional habitats. Maintaining extensive tracts of mature deciduous forests to support the high diversity of mature forest birds, many of which are high conservation concern species, is one of the highest PIF conservation priorities within the EIS study area. We encourage every effort to minimize the removal and fragmentation of existing mature forest habitat within the EIS study area.

19-3-2

----- Forwarded by David Rider/R3/USEPA/US on 01/09/2004 02:51 PM -----

Mark Donham
< markkris@ earthli   To: R3 Mountaintop@ EPA
nk.net>   cc:
                    Subject: Heartwood comments on mountain top removal draft EIS
01/05/2004 08:46
PM

Dear US EPA,

These are the comments of Heartwood regarding the draft EIS on mountain
Top removal (MTR). Heartwood has many members who are directly and
indirectly impacted by MTR.

How can the government let coal companies destroy Appalachia with mining
practices that level mountaintops, wipe out forests, bury streams, and
destroy communities.

| 1-9

According to the administration's draft Environmental Impact Statement
(EIS)
on mountaintop removal coal mining, the environmental effects of
mountaintop
removal are widespread, devastating, and permanent. Yet the draft EIS
proposes no restrictions on the size of valley fills that bury streams,
no
limits on the number of acres of forest that can be destroyed, no
protections for imperiled wildlife, and no safeguards for the
communities of
people that depend on the region's natural resources for themselves and
future generations.   What kind of mitigation is that. In the absence
of
mitigation, the agency must explain in detail what the impacts will be
without any mitigation.

| 1-5

How can relaxing the current regulations protect the environment? The
draft
EIS proposes streamlining the permitting process, allowing mountaintop
removal and associated valley fills to continue at an accelerated rate.
The
draft EIS also suggests doing away with a surface mining rule that makes
it
illegal for mining activities to disturb areas within 100 feet of
streams
unless it can be proven that streams will not be harmed. This
"preferred
alternative" ignores the administration's own studies detailing the
devastation caused by mountaintop removal coal mining, including:

| 1-10

- over 1200 miles of streams have been damaged or destroyed by
mountaintop
removal

- direct impacts to streams would be greatly lessened by reducing the
size
of the valley fills where mining wastes are dumped on top of streams

- the total of past, present and estimated future forest losses is 1.4
million acres

- forest losses in West Virginia and Kentucky have the potential of
directly
impacting as many as 244 vertebrate wildlife species

| 1-5

- even if hardwood forests can be reestablished in mined areas, which is
unproven and unlikely, there will be a drastically different ecosystem
from
pre-mining forest conditions for generations, if not thousands of years

- without new limits on mountaintop removal, an additional 350 square
miles
of mountains, streams, and forests will be flattened and destroyed by
mountaintop removal mining

One thing we want to specifically comment on is any potential "no
jeopardy"
opinions regarding the critically endangered Indiana Bat. We do not
believe
that agencies can justify any more taking of Indiana bats, and that any
taking is jeopardizing the continued existence of the species.

| 8-1-2

These impacts are nothing short of devastating to local neighborhoods and the ecology of the
region. We oppose any decision to continue MTR. This is a barbaric, injust, and destructive
practice that our childrens' children will be paying for. Please stop MTR.

| 1-9

Sincerely,

Mark Donham
Heartwood Program Director
RR# 1, Box 308
Brookport, IL 62910

618-564-3367

Forwarded by David Rider/R3/USEPA/US on 01/08/2004 01:58 PM ----

Jenny Dorgan
<cleanair@aeconli   To: R3 Mountaintop@EPA
ne.ws>   cc:
            Subject: For the People
01/06/2004 10:27
AM

Mr. John Forren,

I am writing on behalf of the Alabama Environmental Council, a statewide
non-profit organization dedicated to protecting environment, citizens
and biodiversity. This purpose of this message is to state our
opposition to mountaintop removal and valley fills and any change in the
rule protecting stream buffer zones.

1-9

It is extraordinarily disppointing that the federal govenrment is
ignoring its own studies by proposing to reduce protections for people
and the environment.

1-10

We ask for a new study that looks at the alternatives to prevent new
mountaintop removal and valley fill operations and to stop the existing
ones within 5 years or by the expiration of the current mining permit,
whichever date occurs first.

1-5

As a government official and a part of the major governing process of
protecting the environment and the citizens of this country, I hope that
you will do your patriotic duty to stand up for what is right and good
for the people.

Jenny Dorgan
Program Coordinator
Alabama Environmental Council, Inc.
2717 7th Avenue South Suite 207
Birmingham, AL 35233
(205) 322-3126

REC'D AUG 0 4 2003.

The Synod of
WEST VIRGINIA-WESTERN MARYLAND
of the
EVANGELICAL LUTHERAN CHURCH IN AMERICA
The Atrium • 503 Morgantown Avenue, Suite 100 • Fairmont, West Virginia 26554-4374

The Reverend Ralph W. Dunkin, Bishop
Email: Ralph.Dunkin@ecunet.org

Phone: (304) 363-4030
Fax: (304) 366-9846

The Season of Pentecost
July 31, 2003

Mr. John Forren
USEPA (3EA30)
1650 Arch Street
Philadelphia, PA 19103

Dear Mr. Forren,

Grace and peace be unto you during this spirit-filled season.

Before the time of public comment on the Environmental Impact Study ends, I wish to make the following comments.

In 2001 devastating rains that resulted in four major floods in this region impacted southeastern West Virginia. In early August of 2001 I toured the flood-ravaged area. People in these areas pointed out the lands that had been "reclaimed" from mountain top and strip mining. My initial observation was that of why were there no trees growing on top of these mountains?

Common sense states that where trees are on top there will be less run off and the chance for fewer floods. Seeds from said trees would naturally flow downward and create new growth. Natives to these regions state that so much ground/dirt has been removed that roots cannot thrive in this poor soil.

17-3-2

Unless the Federal Government works to take care of our own people we will waste billions of dollars on the clean up from floods. The churches of West Virginia have stood by our people. We have re-built homes, cleaned up mud, and sadly moved people out of state.

I am aware that there seems to be a fine line between the creation of jobs and fairness to those who live near the mining sites. There is also a very fine line between clean drinking water and an ecosystem that will be devastated for generations.

Scientific studies have shown that mountaintop removal and valley fills bury and destroy important headwater streams, destroy biological rich forest and stream ecosystems, damage drinking water sources used by millions of people, cause frequent and severe flooding, and harm the quality of life in mountain communities.

9-2-2

9-4-2

Our vision as Lutherans is to be Christ-like servants of hospitality sent to share
God's gift of grace in Jesus Christ in the community of Appalachia.

A layman's reading of the Clean Water Act and Surface Mining Laws not only allows by requires our government to prohibit the use of valley fills and mountaintop removal. Twenty-five years of lax enforcement have created an unacceptable situation. Existing laws should not be weakened, but strenuously enforced.

My prayers are with you and the people who are live daily with your decisions.

Yours in our Lord's service,

Ralph W. Dunkin, Bishop

CC: Carol Warren, West Virginia Council of Churches
Tena Willemsma, Commission on Religion in Appalachia
Danielle Welliever, ELCA Director for Environmental Education
Dory Campbell, Evangelical Lutheran Coalition for Mission in Appalachia

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ARCH COAL, INC.

LAWRENCE D. EMERSON
Director of Environmental Performance

December 17, 2003

Mr. John Forren
US Environmental Protection Agency (3EA30)
1650 Arch Street
Philadelphia, PA 19103

RE: Written Comments on the Draft Mountaintop Mining EIS

Dear Mr. Forren,

In accordance with the press release dated August 14, 2003, please find enclosed two (2) sets of written comments related to the aquatic section of the draft Environmental Impact Statement document. More specifically, these comments are responses to EPA's written comments to our benthic macroinvertebrate report that Arch Coal Inc., conducted within the Mud River, Spruce Fork and Island Creek watersheds located in southern West Virginia.

6-6-5

In the spring of 2002, Arch Coal Inc. submitted to EPA Region III a supplemental quantitative report of benthic studies conducted in the watersheds associated with three our coal mining operations. The studies were based on our own sample collections from the EPA selected sites, using quantitative sampling methods. That report was submitted to EPA for peer review purposes, and the documents submitted herewith are our responses to EPA's comments.

The first document, entitled "Response to US EPA's Comments..." is in a comment and response format. In those instances where EPA's comment resulted in a change in the body of the Arch report, those changes were made and are reflected in the final supplemental report, also enclosed.

Thank you for the opportunity to comment. We look forward to the release of the final EIS document.

Sincerely,

10 Kenton Drive   Charleston, WV 25311   (304) 357-5716   Fax: (304) 357-5725

POTESTA

POTESTA & ASSOCIATES, INC.

Engineers and Environmental Consultants

September 2003

RESPONSE TO UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY'S
COMMENTS ON
"SUPPLEMENTAL QUANTITATIVE BENTHIC MACROINVERTEBRATE STUDIES
IMPLEMENTED IN CONJUNCTION WITH THE USEPA
MOUNTAINTOP MINING/VALLEY FILL
ENVIRONMENTAL IMPACT STATEMENT STUDY WITHIN
THE MUD RIVER, SPRUCE FORK, AND ISLAND CREEK WATERSHEDS"

Prepared for:

Arch Coal, Inc.
10 Kenton Drive
Charleston, West Virginia 25311

Prepared by:

Potesta & Associates, Inc.
2300 MacCorkle Avenue, S.E.
Charleston, West Virginia 25304
E-mail: potesta@potesta.com

Project No. 01-0057-006

Project 01-0057   September 23, 2003

**Response to United States Environmental Protection Agency's
Comments on
"Supplemental Quantitative Benthic Macroinvertebrate Studies Implemented
In Conjunction with the USEPA Mountaintop Mining/Valley Fill
Environmental Impact Statement Study Within
The Mud River, Spruce Fork, and Island Creek Watersheds"**

Prepared by: Potesta & Associates, Inc.

The United States Environmental Protection Agency (EPA) comments are in normal type with the response inserted into the document in **bold** font.

Points where we are in agreement:

1. The filled sites are in worse biological condition than the unmined sites.

2. The filled residential sites are in worse biological condition than the unmined sites.
3. The filled sites represent a wide range of conditions (good to impaired).

4. The filled residential sites are in a narrower range of conditions (impaired).

5. The unmined sites are in a narrow range of conditions (good to very good).

6. Water chemistry is significantly different between classes.

7. Habitat and substrate are not significantly different between the classes.

8. The biological and water chemistry changes are typical of mining impacts.

9. These biological and water quality effects are statistically significant.

10. Sulfate is likely a significant contributor to the high conductivity.

GENERAL COMMENTS:

In general, we disagree with the way water quality issues are treated as an afterthought throughout the report. The report repeatedly infers that temperature, ponds, and stream order are the main contributing factors to the biological condition rather than changes in water chemistry. The report secondarily refers to other factors such as flow, low dissolved oxygen, embeddedness, scouring from flooding, canopy changes from deciduous to evergreen, and the amount of canopy.

The report provides no correlation analyses and, in some cases, no or inadequate data to support these statements, and in some cases, the authors ignore their own statistical analyses where there are relevant data. Our exploratory correlation analyses indicated conductivity (-0.741 for EPA field conductivity) and total dissolved solids (-0.716) had the strongest and most significant relationships to biological condition. Both of these parameters are directly related to mining impacts.

POTESTA: The report does not infer that temperature, ponds, and stream order are the main contributing factors to the biological condition, but does conclude that the effects of these factors cannot, with the data available, be separated from mining effects or effects of valley fills, and that all aforementioned variables are potential contributors to the current in-stream conditions. POTESTA's analysis of the data did not include correlation analysis because there are too many factors not included in the EPA's study to have confidence in the results. For example, the conductivity and total dissolved solids would be higher in areas with more mining activity. These areas would also have more numerous ponds, but may or may not have more numerous or larger valley fills. Under this scenario, it is not clear whether a correlation exists between the biological condition and the area mined, area of the settling ponds, or number and size of the valley fills.

No changes were made to the text as a result of this comment.

The only temperature data offered in the report is the field data for the Winter and Spring of 2000. The statistical analyses of these data indicated there was no significant difference between the site classes. This finding does not support the Potesta conclusions. Even if there were temperature differences Potesta offers no supporting information or data to confirm it. The emergence time issue is not scientifically defensible.

POTESTA: Temperature data available for this study are from two dates in the Spring and Winter 2000 and no significant differences exist between the site classes on these days. However, data from two dates which are not representative of the seasonal temperature variations does not adequately describe what goes on in the system over the course of an aquatic insect's lifecycle. While no information may be specifically available regarding the temperature conditions which occur below valley fills, the temperature differences below impoundments and the impacts to the benthic macroinvertebrate community are well documented. Warmer than normal winter temperatures eliminate

the thermal cues needed for many species to break egg diapause. Cool summer temperatures can result in too few degree-days to complete development. Life cycles can loose their synchrony and impair reproductive success (Allen, 2000). A shift in temperature as small as 2°C to 6°C has been shown to alter life-history characteristics (Ward, 1992). The text will be revised to include a discussion of relevant literature.

If the ponds were the primary factor in determining the benthic community downstream, then we would expect to see similar biological communities downstream of all the ponds but instead the data indicate a range of conditions below ponds. The condition of filled communities in our study ranged from poor to very good in both the Winter and Spring of 2000. The correlation between TOC, DOC, and biological condition was -0.388 and -0.183, respectively. Other parameters, including base cations and metals had higher correlation coefficients than the carbon parameters: e.g. Ca(-0.710), Mg(-0.689), Se(-0.528).

POTESTA: Paragraph 4. The ponds are not indicated to be a "primary factor" in determining the benthic community downstream, but one of several factors which may be influencing the community. This study did not purport to have sufficient information to discern between the potential impacts. That said, the idea that the communities at all sampling locations downstream of the pond should be similar is not plausible. There is no available information on the size or number of ponds upstream of each site, the distance from the sampling location to the pond, whether the pond is surface or bottom release and many other variables. Also, consideration must be given to variables such as water chemistry for which there is some limited information available. The range of conditions which are found to exist downstream of the ponds undoubtedly reflects the range of conditions upstream of and within the ponds.

This report has no biological or chemical data from sites above ponds and in our study we only had two sites above ponds. These sites ranged in condition from fair to good during the Winter and Spring of 2000. If we had more information about the water above the ponds, we would be better able to understand what impact the ponds were having on the streams below the ponds.

POTESTA: Paragraph 5. We are in agreement that more information is needed about the conditions upstream of the ponds. Of the two sites upstream of ponds which were included in the EPA study, one site is apparently bedrock substrate and therefore not comparable to the gravel cobble substrate sampled in free flowing reaches. It is true that if there was more information about the water above the ponds, we would be better able to understand what impact the ponds were having on the streams below the ponds. This variable would have best been considered before the data were collected during the site selection phase.

Stream order is not an issue when comparing unmined and filled sites in this study since sites in both classes were on small, low order streams. All the unmined sites were on first and second

order streams and all but two of the filled sites were on first and second order streams based on 1:24,000 scale maps. In the mountaintop mining area of West Virginia, there are no large streams(third and fourth order) without some type of mining in the watershed. The statistical analyses in the report (Table 19) indicate there is no significant difference between these two classes. These stream orders (1-3) are often included together in index development and often have the same reference condition because in that size range, stream order does not explain a lot of natural variability in the reference sites and the data do not indicate a need for classification to stream order (e.g. the WVSCI, the regional EMAP MAHA and the MD MBSS ffils are for 1-3rd order streams based on a 1: 1 00,000 scale map). Based on your statistical analyses the stream order of the filled/ residential sites are significantly different from the unmined sites. The larger stream size of the filled/ residential sites will mask any potential impairment and not amplify it. These larger streams can appear to be less impaired because they have the potential to contain more taxa than smaller streams.

POTESTA: Stream order is always an issue when selecting sites for comparison and should have been considered prior to study initiation so that appropriate references could have been determined for each stream class. The stream orders from the unmined and filled sites do overlap so there is no statististical difference; however, the differences in the stream sizes should be considered as a potential source of the variability seen in the filled sites. The larger streams in the filled/residential sites are significantly different than the reference streams and are not suitable for comparison to the headwater reaches. To say that such a comparison will "mask impairment" is not a clear representation of the situation. Any changes in community structure, such as those described by the river continuum concept, will show up in data analysis as being a "different" community; which, as has already been established, is then labeled as "impaired". These comparisons are inappropriate and if suitable reference sites were not included in the study it indicates a poor study design, rather than actual impairment.

SPECIFIC COMMENTS

Cover Letter Page 2 -Disagree that the overall difference between the USEPA's two contractor laboratories cause all of the water chemistry data to be called questionable. Blank and duplicate samples provided information regarding the accuracy and precision of the data. In the blank and duplicate data from the second laboratory there is no evidence to suggest that the data from this laboratory is not reliable. We do agree with the following statement "These QA/QC issues do not change the overall conclusion that significant differences exist between the filled and reference (unmined) sites and between the filled/residential and reference sites."

POTESTA: As has been explained to the US EPA personnel previously, the language in the cover letter to which they are objecting was written as a caveat to readers when the revised data set was discovered. At the time, it was not apparent which data used in the original report were acceptable and which were questionable. No changes will be made resultant from this comment.

Page i -We agree with the last sentence in Al Hendricks excerpt.

POTESTA: The last sentence of Al Hendricks review, with which the US EPA agrees, summarized the POTESTA findings.

Page i and ii -Is it possible to see the full comments from the reviewers?

POTESTA: Specific comments from the reviewers were incorporated into the text. General comments from the reviewers are provided.

Page 1, paragraph 4 -See general comments.

POTESTA: See response to general comments.

Page 1, paragraph 4 -The last sentence of this paragraph is clearly speculation and not supported by the data. Our correlation analysis indicates the changes are strongly related to chemistry parameters. The filled /residential sites do have additional stressors in them that the filled sites do not. The filled/residential sites have refuse piles, other mining, larger roads and highways, and residences, all of which can contribute to a more degraded community.

POTESTA: While the reviewer may find the last sentence objectionable, no other explanation is offered for the discrepancy between the "impairment" indicated by the water chemistry and the biological data. The data clearly indicates that if water chemistry alone is responsible for the "impairment" in the biological community, then the filled sites should be more significantly degraded than the filled residential sites. The refuse piles and other mining influences offered as potential additional degradation in the filled/residential sites would have shown up in the water chemistry. The larger roads and highways should have shown up as a significant stressor in the water chemistry (TSS and TDS) and in the embeddedness and habitat evaluation.

The impact of the residences is noteworthy and does show up in water chemistry analysis in the form of nutrients. This is exactly why sites with residential impacts should not be included in the analysis of valley fills and mining without appropriate reference sites.

Page 1, paragraph 5 and continued page 2

The discussion of changes in function and the reliance on functional feeding group indicators is highly suspect since it is well known that it is difficult to correctly assign functional feeding groups at the family level (due to generic differences) and to early instars. More importantly, these types of metrics are almost never chosen for multimetric development for stream assessment they do not adequately discriminate between reference and impaired sites. For example, in the WVSCI report, the following information appears on page 16: % Filterers, the trend was opposite of that expected, interpretation unclear; % Scrapers, poor discrimination; % Collectors, trend opposite from expected, interpretation unclear; % Predators, poor discrimination; % Shredders, skewed distribution, high variance, and marginal discrimination. These metrics are not used because they cannot identify impairment.

POTESTA: Both Merrit and Cummings (1996) and the US EPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (EPA 841-B-99-002) provide functional feeding group information at the family level and while it is more variable than generic level information, it is still valid. Most of the information used in this report and the US EPA's report relative to the benthic macroinvertebrate community structure (i.e. number of taxa, tolerance values, etc.) would be more specific if identifications had been conducted to the generic level. However, the US EPA made the decision that family level data was sufficient for the purpose of this study, and POTESTA is reporting the data to be comparable with the US EPA study.

The use of functional feeding group analysis to document the changes in the benthic macroinvertebrate community as a result of disturbance are widely documented (Camargo and de Jalon, 1995; Poff and Matthews, 1986; Short and Ward, 1980). The data are not included herein as metrics to indicate whether significant changes exist, but as a tool to evaluate the factors contributing to significant changes (already indicated by more traditional metrics). Macroinvertebrate community structural elements (e.g. numbers, taxa, diversity, etc.) often present an incomplete picture of community responses to stress (Barret 1981; Matthews et al. 1982 in Poff and Matthews, 1986). Considering the functional feeding group distribution provides additional insight into the nature of community responses and may reflect altered tropic conditions which can profoundly affect community structure (Poff and Matthews, 1986). In this manner, the functional feeding group information serves in a similar manner to the habitat data and the water chemisty in providing information on factors contributing to the changes in the biological community. The reviewer appears to have misunderstood the

intent of the discussion. A discussion of the intent of the analysis has been added to the text for clarification.

If we did make a big assumption and say they did work, then the first and last sentence of this paragraph do not fit in with your own statistics. The first sentence states no significant adverse impacts and the last sentence states stream function does not appear to be compromised. In looking at your own statistics, there are significant differences between the stream classes for both the spring and winter sampling seasons. This would indicate that functional feeding groups are being impaired or compromised at the filled and filled/residential sites. The fact that they are all represented does not mean they are in good condition.

POTESTA: As stated above, there is no need for an assumption that functional feeding group metrics "work" in this analysis. The first sentence in the paragraph states that there appears to be no significant adverse impacts on the stream function with respect to downstream segments. This does not contradict the finding of statistical differences in the biological community. Stream function refers to the ability of the stream to support a benthic macroinvertebrate community, process nutrients in different forms, and provide nutrient sources to downstream communities. The functional feeding group analysis indicates a shift in the community which indicates differences in food supply; however, the stream function is preserved. Failure of the community to utilize an available food source (i.e. loss of a functional feeding group) or failure to respond to a shift in available food sources would indicate lack of stream function. A significant difference in the functional feeding groups between unmined and filled or filled/residential sites does not indicated "impairment". It indicates an abundance of some other type of food source, which is being utilized by the community. This is exactly the type of information a researcher hopes to find when trying to determine factors contributing to the significant differences seen in the community metrics. There will be no change in the text in response to this comment.

Page 2, paragraph 2

The changes in water quality and biological communities below the fills is related to the entire mining operation (the mined area above the fill, the fill, the roads associated with the mining, and the sediment ponds). But, the one fact that cannot get lost, that is directly associated with the fills, is direct stream loss under the fills.

POTESTA: The objective of this study was to determine effects of valley fills on the biological community downstream of the fill. This is why all the study sites were located downstream of the filled areas. Stream loss under a fill is not a focus of this particular study. We appear to be in agreement that changes in water quality and biological communities below the fills are related to the entire mining operation (the mined area above the fill, the fill, the roads

associated with the mining, and the sediment ponds) and fill effects cannot be specifically differentiated with the current study design.

Page 2, paragraph 3

Stream Order: See general comments.

POTESTA: As stated previously and in the text of the report, the changes associated with increasing stream order should have been considered in the study design phase and should certainly be considered in the data interpretation. There will be no change in the text in response to this comment.

Page 5, Section 2.2.1 and 2.2.2

It should be noted that although many of the unmined sites could not be sampled during the summer and fall of 1999, they were not all necessarily dry. When these streams were sampled the following winter they were all in good or very good condition. That indicates that even though there may not have been any visible surface flow or not enough surface flow to collect a representative sample, the invertebrates were still there. Many of these streams did have perceptible surface flow, they definitely had subsurface interstitial flow, and many had residual pools. The macro invertebrates had refugia during the drought. We just could not sample them.

POTESTA: The report text is changed to reflect little or no flow creating conditions which prohibited sampling.

Page 8, 2.6 Bioassessment Metrics
There should be some better justification for metric selection other than " the standard metrics that Potesta uses". Is there some background work or documentation that has been done to justify their selection? Generally metric are selected based on discrimination ability, variability, and redundancy. Has any of this been done? This section needs beefed up.

POTESTA: The metrics selected for use in the bioassessment were selected by Dr. Frank Borsuk based on guidance by the US EPA's bioassessment methods document. It is acceptable to use metrics suggested by the US EPA without discriminatory analysis on every study because the discriminatory ability has been tested in a wide range of conditions by the US EPA or (or other researchers and presented in the EPA document) prior to the presentation of the metrics in the RBP protocol. Additionally, multiple metrics are presented with benefits and limitation of each so that professionals can use their judgment in selecting an array of metrics for use in a particular study. A reference to the US EPA document used in the metric selection has been added to the text.

Functional feeding groups are used in the report, but there is no write up in this section justifying their use and the importance of using them. There is also no discussion how each taxa was assigned to a group and there is no list of the taxa assignments.

POTESTA: A discussion of the intent of the functional feeding group analysis has been added to the text for clarification. A discussion regarding group designations and a table showing the functional feeding group classification for each family has also been added to the text.

Page 10, 3.1

"The impacts that the drought in 1999 had on the reference streams are unknown." This is not a correct statement. All the streams were sampled in the winter and spring of 2000, and all were in good or very good condition.

POTESTA: Sampling of the reference streams in Winter and Spring 2000 gives an indication of the condition when the streams were sampled, good or very good. However, this does not give any indication of the impacts that the drought had on the stream communities. Effects of drought on benthic macroinvertebrate communities are well documented and include decreased abundance, increased intra and inter specific competition and predation, an initial increase in taxa richness during the recolonizing period, changes in community structure resultant from alteration in food availability, and water chemistry changes (dissolved oxygen, temperature and other changes associated with slower flow) (Lake, 2000; Allen, 2000). The sampling conducted to determine that the communities were "good or very good" were qualitative and would not indicate a decrease in abundance. They in no way accounted for community level changes from increased intra and inter specific competition and predation or changes in community structure resultant from alteration in food availability. The effects on taxa richness are also unknown because there is no "pre-drought" data available for comparison. The statement that the impacts of the drought on the reference streams is unknown will not be changed in the text.

Page 11, paragraph 2
"Also noteworthy is the increase in filter- collectors in the filled/residential groups, which could be attributed to the organic levels from domestic inputs." The numbers in the table indicate 20.56 % of the individuals in the filled/residential sites were filter-collectors and 20.07 % were filter-collectors at the filled sites. If this is true, where did the nutrients come from in the filled sites?

POTESTA: Not including a discussion of filter-collector increases in the filled sites was an oversight and has been corrected. The nutrient source for the filter feeding organisms is the ponds themselves. Their contribution of a nutrient rich food source and the subsequent increase in collectors is well documented (Stanford and Ward, 1979; Petts, 1984; Allen, 2000).

Page 13, paragraph 1
See previous comments concerning post drought condition of unmined streams. There is no data to support the comment about temperature and D.O. having an influence on the communities. Our D.O. data did not indicate a problem.

POTESTA: This statement is from *Stream Ecology: Structure and Function of Running Waters* (Allen 2000), a stream ecology textbook. The author is relying on a basic knowledge of stream dynamics that the reviewers were believed to share. Not only was 1999 a drought year, but also one of the hottest years on record. Under drought conditions, flows are reduced. The reviewer has stated that flow was negligible, often subsurface and in some places only pools remained for refugia for the organisms. Without measuring, it is safe to assume that the more water you have, the less likely it is to respond to temperature fluctuations in the environment. Subsequently, the less water available, the harder it is to maintain water temperature in the stream and the greater are temperature fluctuations. It is well documented that dissolved oxygen is inversely related to temperature. So, with high temperatures (such as those reported during one of the hottest years on record), dissolved oxygen saturation would have been reduced. Since the most reaeration occurs in riffles and under flowing conditions, the low flow conditions (as stated by the reviewer) would not have been conducive to rearation. Also, organic material in the sediments and in pools exerts an oxygen demand not present in riffle/gravel/cobble substrates which would further add to the oxygen demand. The reviewer states that their data did not indicate a dissolved oxygen problem; however, the author would not expect dissolved oxygen readings taken during the daylight hours to necessarily reflect a problem. These data would represent one instance in time, and not the conditions to which the organisms are exposed. An analogy would be to sample the organically rich area below a waste treatment plant on a warm summer afternoon when the water is supersaturated with oxygen ignoring the diurnal fluctuations and nighttime sag and stating that DO is not a problem. A researcher has to interpret data using all the information at their disposal. A discussion is included in the text describing the impacts of drought on streams and biological communities.

Page 13, paragraph 2
The term "moderate richness and abundance" is used in this paragraph. What is it moderate in relationship too?

POTESTA: The terms "moderate richness and abundance" and "low richness and abundance" are both used in this paragraph. They are subjective terms, which refer to low levels and medium levels of richness and abundance based on the other sampling locations used in this study and the researcher's knowledge of the communities expected to be present under ideal conditions in the streams. No change has been made to the text as a result of this comment.

Page 13, paragraph 3
"Chironomidae, another filter feeder". Is this the group you put them in or is this a mistake?

POTESTA: Chironomidae are collector-gatherers and were placed into this category for functional feeding group analysis. The text has been changed to reflect the collector-gatherer category.

Page 13, 4.2 Winter Benthic Macroinvertebrates

The abundance at the unmined sites was not significantly different from the filled sites but the filled residential sites were significantly different from the unmined sites. Higher abundance is not an indicator of better conditions, it is generally an indication of impaired condition. The condition of the benthic community by site class indicates the unmined sites are in the best condition, followed by filled sites and then the filled/residential sites. The abundance data would put them in the same order which clearly indicates that more is not necessarily better.

POTESTA: Abundance data can either increase or decrease in response to stress. While it can indicate enrichment of a food source, as in the filled/residential sites, it can also indicate impairment. Reduced abundance is associated with recovery from drought conditions and it is the professional judgment of the researcher that an average of only 100 organisms in a surber sample is on the low side. There is no indication that the unmined sites are "better" than the filled sites with respect to abundance. No changes will be made in the text.

Page 14, paragraph 1

Some stoneflies are tolerant to the constituents found in mine drainage and acid rain impacted streams. Mayflies on the other hand are not. The statement that water quality may not be the limiting factor is rather erroneous. True, they are both sensitive orders but they can be sensitive to different constituents.

POTESTA: According to the RBP, the tolerance values of mayflies range from 0 to 9 while the tolerance values of stoneflies ranges from 0 to 6.3, indicating that both groups of organisms are similar in their sensitivities. While it is true that some stoneflies have been found to be somewhat tolerant to mining related discharges, the number and diversity of stonefly taxa present and the discrepancy between the water chemistry and biological data still indicate that more information is needed to determine that water quality is the limiting factor in the streams. No change is made in the text in response to this comment.

Page 14, paragraph 2
The report indicates that the characteristics of the fills might explain the variability in the biological communities. The report also lists many of the things that can affect the fills but does not state that all these things will also have an impact on the water quality exiting the sediment pond. In our report, the range of biological conditions was best explained by water quality.

POTESTA: The paragraph in the text has been expanded to include a discussion of several other factors which may be contributing to the variability seen in the

filled sites. The author disagrees that in the US EPA report the range of biological conditions was explained by water quality. The US EPA report failed to consider significant sources of variability and relies on correlation analysis without taking into account the potential for alternate correlations with the variables they ignored. "The presence of a correlation between two variables does not necessarily mean there exists a causal link between them." (Glass and Hopkings 1984)

Page 14, paragraph 3

"The algae and detrital material flowing from the ponds acts as the food source for the downstream communities." We are not pond experts but would think that ponds would be detrital sinks not a source.

POTESTA: The lentic system can act as a detrital sink, but they are also a source. While much of the productivity comes from photosynthesis of algae, this is dependent on the rich nutrient source of detrital breakdown. However, "detritus includes particulate and dissolved organic carbon..." (Smith 1992) which is discharged via the outfall. This reference is a general ecology text book.

The statement, "Since this is a more continuous and less variable food supply than leaf litter", has nothing to support it. There is no data in the report and no references to defend this statement. We did not measure in stream leaf litter but our visual observations and photographic record indicate there is leaf litter in these streams below the ponds.

POTESTA: The potential changes below impoundments include reduced variability in thermal regime, food quality and quantity, flow conditions, and other parameters which are well documented in the literature (Stanford and Ward 1979; Petts 1984; Kondratieff and Voshell 1980). A photographic record of leaf litter does not indicate the quality or quantity of a food supply. The availability of the food source is related to many variables.

"While this represents a fundamental shift in the biological community, the community created is not necessarily undesirable," The Clean Water Act was written to protect biological integrity and integrity is defined as an unimpaired condition not a changed condition.

POTESTA: The goal of the Clean Water Act is to "restore and maintain the chemical, physical, and biological integrity of the Nation's water." The author is unaware of any place in the Clean Water Act where biological integrity is defined or where "change" is defined as impairment. The reviewer should provide a reference for that interpretation. If that is the case, than any dam constructed for any reason (flood control, hydroelectric power, sediment retention, recreation) would be in violation of the Clean Water Act, as would be many other activities which are currently permitted or acceptable practices.

Page 15, paragraph 1
The only habitat data we observed in the report was ours and that embeddedness data did not indicate a problem with the filled sites. If there is data out there that can support the statements about embeddedness, increased flooding and scouring, or changes in the type and amount of canopy cover in the filled sites it should be in the report or these speculative statements should be dropped from the report. If there is increased flooding and scouring below the mines it would not be good news for the industry.

POTESTA: Changes in sediment deposition from mining, timbering, road construction, and other development are widely documented. It is somewhat of a surprise, and a testimony to the effectiveness of the sediment control structures (ponds), that embeddedness was not significantly higher in the mining influenced sites in this study. However, embeddedness has been removed as a potential variable contributing to scraper declines in mining influenced streams. Changes in the flow regime below mine sites are not news to the industry. As required by regulation, specific steps are taken on mine sites to move water quickly away from areas of overburden storage where infiltration may lead to saturation and potential stability problems. The direction of water away from these areas, and the movement of water through these areas, results in hydrographs very different from a natural stream. The presence of a pond further alters the hydrograph of the downstream reaches. Care is taken during the planning stages of mining activities to ensure that stream channels are capable of receiving the flow magnitude and velocities generated on the sites. Depending on the site conditions, increased peak discharges and scouring in a downstream reach are possible, as are lower flow conditions in a stream reach. The "speculative" statements will not be removed from the report. They are, in the best professional judgment of the author, plausible explanations for variability seen in the data and perfectly appropriate for the discussion section of a scientific study.

Page 15, paragraph 3
Simuliidae filter FPOM with fans, they do not siphon water.

POTESTA: The text has been clarified.

Caddisflies are ubiquitous except in the most toxic conditions, so to say they are found below ponds and waste treatment plants is not news; they are found everywhere.

POTESTA: While caddisflies are ubiquitous, the point of the discussion is that they occur in increased abundance and are often the dominant organism in communities below ponds and waste treatment plants, a condition found in the current study. The importance of the shift of the benthic community to one comprised of 75% collectors has been clarified in the text for the reader.

Page 16, paragraph 1
There are no data to support the temperature data. See previous comments.

POTESTA: See response to general comments.

Page 16, paragraph 2
The increased alkalinity is not "a significant benefit to the streams." These streams are naturally low in alkalinity and conductivity and support diverse macro invertebrates community. To suggest that the water quality is improved below the filled sites totally ignores the biological data. Again, there is no data to support the statement "acidic precipitation could cause excursions of the pH below the acceptable level." We observed no indications of a problem.

POTESTA: The EPA's April 8, 2002 document entitled "A survey of the Water Quality of Streams in the Primary Region of Mountaintop/Valley Fill Coal Mining" states that the only pH excursions below the 6.0 SU water quality standard were in unmined streams and "could be a result of acid deposition" (Page 73). The previous statement that no indications of a problem were observed is incorrect. Also, POTESTA's analysis of the field data indicated significant differences between the unmined and filled sites with the unmined sites having pH values lower than the filled sites. Acid precipitation is increasing globally (US EPA Acid Rain Program Website), as most scientists are aware. West Virginia is in an area of increasing acid deposition as indicated by the isopleth diagrams from 1994 and 2000 (attached). In 1998, West Virginia's 303-d list was expanded to include a number of streams listed as impaired due to acid precipitation. While atmospheric deposition is not listed on the 2000 303-d list, due to the uncertainty from mining influences and the naturally acidic conditions of some streams, it is still considered to be a limiting factor in some streams both locally and globally. Further, due to leaching of the buffering capacity of soils and the continued decline in precipitation pH, the acidification of streams related to acid rain is not expected to decline in the near future. It is the judgment of the author that the increased alkalinity is a benefit to the streams. The text was not modified in response to this comment.

Page 16
There is no mention of the Selenium criteria violations. Is it because the data was not available at that time?

POTESTA: Selenium criteria violations were noted in the unmined, filled and filled/residential streams in the water chemistry samples analyzed in this study. Although the water chemistry data were revised to remove all samples not passing quality assurance testing, the values from the Winter and Spring 2000 data are still higher (often an order of magnitude) than the second EPA contractor laboratory. Given these discrepancies, both datasets are of little value for comparison to water quality standards until one dataset

can be shown to be accurate. As such, selenium is used only for relative comparisons between the three treatments.

Page 16, last paragraph
The report acknowledges here that there were few habitat differences among the site classes and embeddedness was not one of them. See previous comments for page 15.

POTESTA: See response to comment on Page 15, Paragraph 1.

Page 17, paragraph 1
See previous comments on stream order.

POTESTA: See response to general comments.

Page 17, paragraph 2
Again, increased abundance is a classic indication of stress, as competition decreases from the loss of intolerant organisms there is an increase in the number more tolerant organisms. This is well documented in the literature. Small headwater streams, such as these, with low alkalinity and low conductivity tend to have low numbers of macroinvertebrates. The discussion about the emergence times of the stoneflies is speculation and is not supported by data or literature review.

POTESTA: As indicated previously, abundance can either decrease (as in response to flooding or drought) or increase (as in response to an organic food source) in response to perturbation in a stream. A change in either direction is an indication of stress. The reduced condition is well documented in the literature, particularly with respect to the recovery period of benthic communities following flooding events (Lake, 2000). The increase in abundance in response to organic inputs is also well documented (Allen, 2000). The shift in community structure from an intolerant to a tolerant community described above is not generally accompanied by an overall increase in abundance (rather a replacement) unless an additional food supply is available.

The dependence of the development and emergence time of stoneflies on temperature is well known, as are the responses of the Plecopterans to both "winter warm" and "summer cold" conditions which may prevail below impoundments (Stanford and Ward, 1979). The discussion in the text regarding the effects of valley fills and ponds on stonefly populations is a plausible explanation for the variability seen in the study and is appropriate for the discussion section of the study. No changes have been made to the text as a result of this comment.

Page 17, paragraph 3 and top of page 18
The statement, "decreased scraper community in the spring when leaf cover shades the stream", cannot be documented. We did not do any canopy measurements and we do not see any data to indicate Potesta did either. We sampled in late April and early May before leaf out was complete.

POTESTA: Samples were collected January 21-31, 2000 (Winter) and May 17-18, 2000 (Spring). Although specific measurements were not taken, common sense would dictate that the tree cover in headwater streams would differ substantially between these two periods. That lacking, the attached photographs support increased shade during the spring sampling event (Attachment 2). No changes have been made to the text as a result of this comment.

Page 18, paragraph 1
There is no data or supporting literature to back up the idea that there is a greater food supply for collectors in the streams below fills and ponds.

POTESTA: While the scientific knowledge is limited regarding conditions below fills, there is no shortage of information regarding the conditions below impoundments and pond discharges. In general, an increased density, primarily of filter feeders and collectors is expected resulting from flow constancy, organic loading, or both (Stanford and Ward, 1979; Petts, 1984; Allen, 2000). Although it should be noted that the responses of benthic communities to impoundments are highly variable depending on such factors as release location (surface or bottom release), impoundment size and retention time, water quality, geographic location, and many others. A discussion of the changes in the benthic macroinvertebrate community below impoundments has been added elsewhere in the text.

Page 20
Both the structure and function of streams below valley fills have been altered and as such would not meet the objectives of the Clean Water Act.

POTESTA: The changes in an aquatic system downstream of an impoundment are well documented (Allan, Ward and Stanford, 1979, Petts, 1984, Allen, 2000). If the Clean Water Act (Act) is interpreted such that "restoration and maintenance of chemical, physical and biological integrity" means no change is acceptable below an impoundment, than there are many impoundments created for flood control, hydroelectric production, drinking water reservoirs and beaver housing which are also in violation of the Act. Additionally, many other activities such as removing canopy cover, dredging a channel, building in a watershed, and others, would also be a violation of the Act. The discharge of organic material from a waste treatment plant, while within the permit limits, increases the filter feeding organisms below the discharge and this too would be a violation of the Act. We disagree with the conclusion that because streams are "altered" the activities do not meet the objectives of the Act and would request that the reviewer provide documentation for this interpretation.

Page 16 of 16
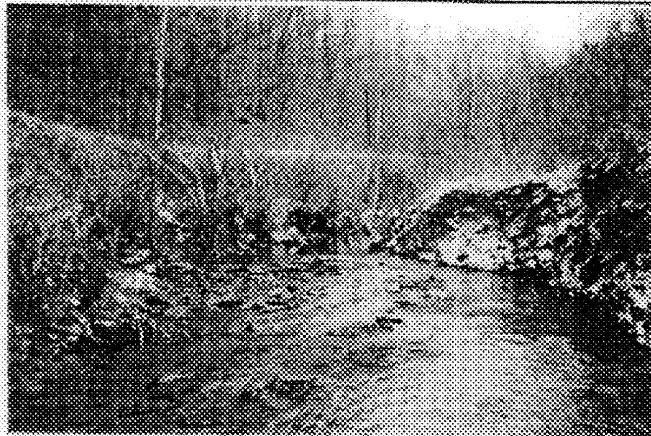

MT-2 Rushpatch Branch Upstream View, January 2000

MT-2 Rushpatch Branch Downstream View, January 2000
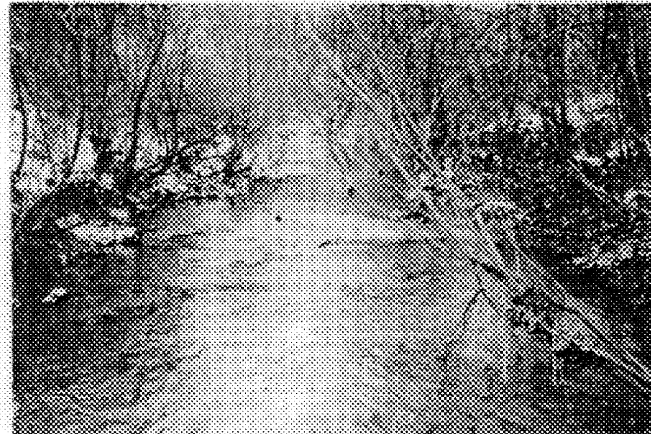
Potesta & Associates, Inc.
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Arch Coal, Inc.
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Project No. 99898-003

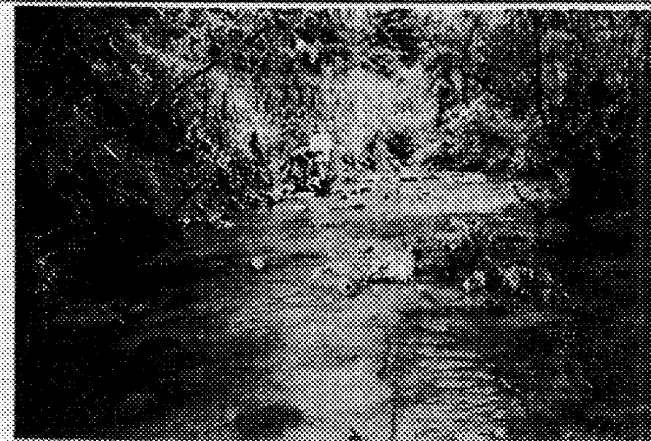MT-23 Mad River Downstream of Connelly Branch Upstream View, January 2000

MT-23 Mad River Downstream of Connelly Branch Downstream View, January 2000
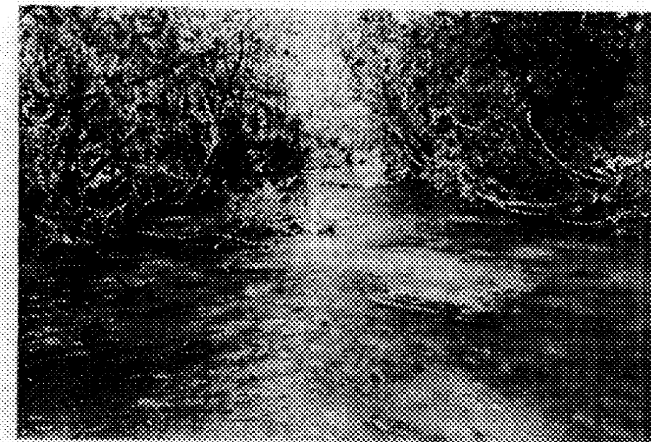
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MT-23 Mad River Upstream View, May 2000

MT-23 Mad River Downstream View, May 2000
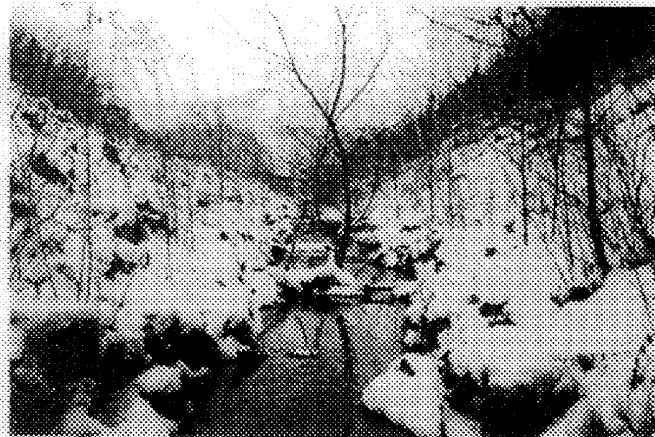
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MT-32 Beech Creek Downstream of Peats Branch Upstream View, January 2000

MT-32 Beech Creek Downstream of Peats Branch Downstream View, January 2000

MT-02 Rushpatch Branch Upstream View, Spring 2000

MT-02 Rushpatch Branch Downstream View, Spring 2000

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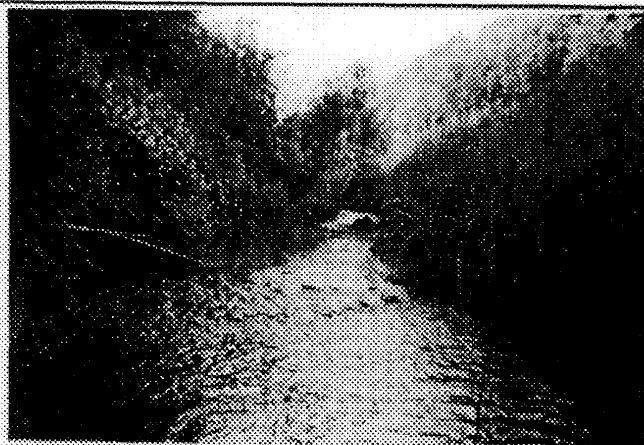| Potesta & Associates, Inc.	Arch Coal, Inc.
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MT-32 Beech Creek Upstream View, May 2000

MT-32 Beech Creek Downstream View, May 2000

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◢POTESTA

POTESTA & ASSOCIATES, INC.

Engineers and Environmental Consultants

September 2003

**SUPPLEMENTAL QUANTITATIVE BENTHIC
MACROINVERTEBRATE STUDIES
IMPLEMENTED IN CONJUNCTION WITH THE
USEPA MOUNTAINTOP MINING/VALLEY FILL
ENVIRONMENTAL IMPACT
STATEMENT STUDY
WITHIN THE MUD RIVER, SPRUCE FORK, AND
ISLAND CREEK WATERSHEDS**

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STUDIES IMPLEMENTED IN CONJUNCTION WITH THE
USEPA MOUNTAINTOP MINING/VALLEY FILL
ENVIRONMENTAL IMPACT STATEMENT STUDY WITHIN THE
MUD RIVER, SPRUCE FORK, AND ISLAND CREEK WATERSHEDS**

1.0   EXECUTIVE SUMMARY

Arch Coal, Inc. (ARCH) acquired the services of Potesta & Associates, Inc. (POTESTA) to collect supplemental benthic macroinvertebrate samples in conjunction with the United States Environmental Protection Agency (USEPA) during the implementation of the Summer 1999, Fall 1999, Winter 2000, and Spring 2000 index periods of the Mountaintop Removal/Valley Fill Mining Environmental Impact Statement Study (MTR/VF-EIS) within the Mud River, Spruce Fork, and Island Creek watersheds. POTESTA collected six supplemental quantitative Surber samples at each monitoring station sampled by the USEPA (except MT-24 which was a wetland-type habitat) during each of the four index periods.

This report is a presentation of the benthic macroinvertebrate data at the familial level. Also incorporated are water chemistry and habitat data collected at the sites by the USEPA. In sampling seasons, when sufficient data were available, statistical comparisons were made between the unmined (reference), valley filled and valley filled/residential sampling sites.

The majority of the reference streams within the three watersheds were dry during the summer and fall index periods. Six of the seven unmined reference streams within the three watersheds were dry during the summer index period. All seven reference streams were dry during the Fall 1999 index period. In contrast, all monitoring stations associated with valley fills had flowing water in the Summer1999 period, and all but one of the monitoring stations had flowing water in the Fall 1999 index period. All 22 monitoring stations had flowing water during the Winter 2000 index period.

Significant differences were seen in both the benthic community and water chemistry between the unmined streams and the filled and filled/residential sites. Differences between the unmined streams and the filled streams may be related to differences in temperature regimes (and therefore emergence times), the presence of ponds (additional food source), and water chemistry differences between the treatments. One interesting finding is that while the most significant biological impairment was indicated in the filled/residential sites, as compared to the unmined sites, the most significant differences in water chemistry were seen between the filled sites and the unmined sites. This indicates that the significant changes in the communities at the filled/residential sites (and possibly the filled sites) results from some variable other than water chemistry parameters.

Neither the changes in the biological community, nor the changes in the water chemistry in the filled sites appear to have significant adverse impacts on the stream function with respect to downstream segments. The most significant changes in stream biological community are the shifts in the functional feeding groups toward more filter feeding organisms and the reduction of the mayfly

community in filled and filled/residential sites. The changes in community structure likely result from the presence of ponds and changes in temperature regimes. This typically occurs in streams whenever ponds, dams or municipal discharges are present. The reduced mayfly populations in the filled and filled/residential sites are not uncommon in areas with mining influence or below impoundments. Although a reduction in mayfly populations is often attributed to the presence of metals, the contribution of sulfate and other dissolved ions may also be important. Increased abundance at the filled sites, as compared to the unmined sites, and the presence of a similar shredder community indicates that sufficient food is available to support a benthic community at these locations and that downstream communities are likely receiving particulate organic material from these more upstream segments. Filled sites and filled/residential sites did not always have identical functional feeding group distribution. For example, a higher percentage of collector-gathers were found below filled/residential sites. The reduction of the mayflies does not appear to affect the function of the streams. Sites influenced by mining continue to support an abundant population with representatives of all the functional feeding groups, and stream function does not appear compromised at these sites.

The changes in the benthic macroinvertebrate communities and water chemistry at the filled and filled/residential sites are consistent with expected changes in any mining influenced streams. These potential changes are related to mining in general, not necessarily to the practice of valley fill construction. Of the changes in both the water chemistry and biological communities which are described in this report, none can be attributed to the fill specifically, and all potentially result from coal mining, road construction or residential development. Additionally, the same changes in water chemistry and biological communities result from large scale development projects and ore extraction and processing operations (ore and gold extraction, steel mills, smelters).

Another consideration in this study is the imbalance in comparing a mined site on a third, fourth or fifth order stream with an unmined site on a first or second order stream. No unmined sites were selected on third, fourth or fifth order streams. Although not necessarily an objective of this study, changes in water chemistry and biological communities between first or second order streams and third or fourth order streams are expected (Vannote et al 1980). The changes associated with increasing stream order should be considered in the data interpretation.

2.0   INTRODUCTION

Arch Coal, Inc. (ARCH) acquired the services of Potesta & Associates, Inc. (POTESTA) to collect quantitative benthic macroinvertebrate samples in conjunction with the United States Environmental Protection Agency (USEPA) during the implementation of the Summer 1999, Fall 1999, Winter 2000, and Spring 2000 index periods of the Mountaintop Removal/Valley Fill Mining Environmental Impact Statement Study (MTR/VF-EIS) within the Mud River, Spruce Fork, and Island Creek watersheds.

The USEPA survey established monitoring stations on the mainstem of the major receiving streams that bracketed the historical and current mining activities. They proposed to assess the biological

condition of the streams with the use of the semi-quantitative kicknet sampling technique at each of the monitoring stations and the use of the quantitative Surber (1 square foot area) sampling technique at selected monitoring stations. POTESTA recommended the collection of six quantitative Surber samples at each monitoring station to improve the statistical power of the analyses.

The USEPA established 23 monitoring stations within the Mud River, Spruce Fork, and Island Creek watersheds (Table 1). Kicknet samples were collected from each of the 23 monitoring stations and Surber samples were collected from selected sites for the EPA study. POTESTA collected six supplemental Surber samples from each site where the USEPA collected a benthic macroinvertebrate sample. The supplemental surber samples were collected during the same time frame as the USEPA studies. Efforts were made to collect samples in the Summer 1999, Fall 1999, Winter 2000 and Spring 2000 sampling seasons. Due to the drought conditions of 1999, several of the study streams were dry and benthic macroinvertebrate samples were not collected in these streams in the summer and fall sampling periods. Supplemental surber samples were not collected from MT-24 because the site was located within a drainage ditch/wetland that was not conducive to quantitative Surber sampling.

POTESTA independently analyzed the quantitative data using the EPA collected water chemistry and habitat evaluation data from the sampling sites. The data were analyzed statistically comparing the EPA identified categories or "treatment" groups of sites which were unmined or reference, sites which were influenced by valley fills, and sites influenced by both valley fills and residential areas. Other groups, such as sites influenced by mining but not valley fills, and sites in sediment control structures were not included in this analysis due to low replication that prohibited statistical analysis. Benthic macroinvertebrate data were summarized and analyzed using metrics indicative of biological condition. Also, differences in the benthic communities were evaluated using a comparison of functional feeding groups to assess the nature of the community changes indicated by the statistical analysis. While changes in functional feeding groups have not consistently proven to be discriminative metrics useful for identifying changes in benthic community structure, consideration of the functional feeding groups distribution provides additional insight into the nature of community responses (Poff and Matthews, 1985) and is a useful tool in evaluating the potential causes of community level changes.

3.0   METHODS

3.1   Study Areas

The USEPA established 23 monitoring stations within the three watersheds as part of the MTR/VF-EIS study (Table 1). Nine monitoring stations were established within the Mud River watershed (Figure 1), eight monitoring stations within the Spruce Fork watershed (Figure 2), and six monitoring stations within the Island Creek watershed (Figure 3). Figures 1, 2, and 3 are copies of USEPA documents showing their selected monitoring stations are used with the permission of the agency. The monitoring stations were designated by the USEPA as either unmined (reference) stream segments, or stream segments with valley fill mining (filled). The filled category was further divided into filled with no residential impacts and filled with residential impacts (filled/residential).

Additional samples were collected in areas that had historical mining with no valley fills (mined) or were historically mined with residential areas. These data are not discussed herein because the sample sizes were so small that they could not be included in the statistical analysis. They are, however, included in the lists of samples collected.

In addition, the USEPA sampling program included sampling locations selected to indicate cumulative mining impacts in the watershed and reference locations were selected for each downstream sampling location. It was later determined by the USEPA that the impacts of mining could not be separated from other multiple influences in the watersheds (Memorandum: From Rebecca Hanmer, January 8, 2001). Therefore, a discussion of cumulative impacts is not included in this report.

3.1.1   Mud River Watershed

The USEPA established three reference stream segments, one mined stream segment, and four filled stream segments within the Mud River watershed. The three reference stream segments were located on Rushpatch Branch (MT-02), Lukey Fork (MT-03), and Spring Branch of Ballard Fork (MT-13). The mined stream segment was located on the upper Mud River (MT-01). Although MT-01 was sampled, the data were not included herein because the sample sizes were too small. The four filled stream segments were located on Ballard Fork (MT-14), Stanley Fork (MT-15), Sugartree Branch (MT-18), and the lower Mud River (MT-23). The lower Mud River, MT-23, was a filled/residential stream segment. The USEPA also established a second mined stream segment within the sediment control drainage ditch at the headwaters of Stanley Fork (MT-24), but POTESTA did not sample this site.

3.1.2   Spruce Fork Watershed

The USEPA established two reference stream segments, one mined stream segment and five filled stream segments within the Spruce Fork watershed. The two "reference" stream segments were located on White Oak Branch (MT-39) and Oldhouse Branch (MT-42). The mined stream segment was located on Pigeonroost Branch (MT-45). Although MT-45 was sampled, the data is not presented in this report. The five filled stream segments were located on Rockhouse Creek (MT-25B), Beech Creek (MT-32), Left Fork of Beech Creek (MT-34B), Spruce Fork (MT-40), and Spruce Fork (MT-48). The two Spruce Fork stream segments, MT-40 and MT-48, are also influenced by residences and are therefore considered filled/residential.

3.1.3   Island Creek Watershed

The USEPA established two reference stream segments, one mined stream segment and three filled stream segments within the Island Creek watershed. The two "reference" stream segments were located on upper Cabin Branch (MT-50) and the lower Cabin Branch (MT-51). The three filled stream segments were located on Cow Creek (MT-52), Hall Fork of Left Fork of Cow Creek (MT-57B), and Left Fork of Cow Creek (MT-60). The Cow Creek station MT-55 was filled/residential.

3.2 Sampling Seasons

As part of the MTR/VF-EIS study, the USEPA sampled over five seasons (Spring 1999, Summer 1999, Fall 1999, Winter 2000 and Spring 2000). POTESTA collected quantitative benthic macroinvertebrate samples over four seasons (Summer 1999, Fall 1999, Winter 2000, and Spring 2000) within the Mud River, Spruce Fork, and Island Creek watersheds. The Summer 1999 studies were implemented during late July 1999, the Fall 1999 studies were implemented during late October 1999, the Winter 2000 studies were implemented during late January 2000, and the Spring 2000 studies were implemented in mid-May 2000.

3.2.1 Summer 1999

Sampling during the summer season was implemented within the three watersheds from July 27 to July 29, 1999. Drought conditions existed during this collection period. POTESTA collected benthic macroinvertebrate samples from four of the nine sampling stations within the Mud River watershed, seven of the eight monitoring stations within the Spruce Fork watershed, and four of the six monitoring stations within the Island Creek watershed.

Within the Mud River watershed, the three unmined monitoring stations (MT-02, MT-03, and MT-13) did not have sufficient flow to collect representative samples during late July 1999, and benthic macroinvertebrate samples were not collected from these monitoring stations. In addition, POTESTA did not collect benthic macroinvertebrates from the drainage ditch (MT-24). Quantitative benthic macroinvertebrate samples were collected from three filled monitoring stations (MT-14, MT-15, and MT-18) and the filled/residential site, MT-23.

Within the Spruce Fork watershed, one (MT-39) of the two unmined stream segments was dry. The second unmined stream segment (MT-42) exhibited low flow conditions. However, POTESTA was able to collect samples at this site. Macroinvertebrate samples were also collected from the filled stations MT-25B, MT-32, and MT-34B, as well as the filled/residential sites MT-40 and MT-48 and the mined site MT-45.

Within the Island Creek watershed, benthic macroinvertebrate samples were not collected from the unmined sites, MT-50 and MT-51, due to dry conditions. Benthic macroinvertebrate samples were collected from the filled stations MT-60, MT-57B, and MT-52 and from the filled/residential site MT-55.

3.2.2 Fall 1999

Sampling during the fall season was implemented within the three watersheds from October 26 to October 28, 1999. All of the unmined streams were dry during the fall sampling season. POTESTA was able to collect benthic macroinvertebrate samples from five of the nine sampling stations within the Mud River watershed, five of the eight monitoring stations within the Spruce Fork watershed, and four of the six monitoring stations within the Island Creek watershed.

Within the Mud River watershed, the three unmined monitoring stations (MT-02, MT-03, and MT-13) did not have sufficient flow to collect representative samples during late October 1999, and benthic macroinvertebrate samples were not collected from these monitoring stations. POTESTA did not collect quantitative samples from the drainage ditch (MT-24). Benthic macroinvertebrate samples were collected from the filled sites MT-14, MT-15, and MT-18. In addition, benthic macroinvertebrate samples were collected from the filled/residential site MT-23. A sample was also collected from the mined site MT-01.

Within the Spruce Fork watershed, both unmined monitoring stations (MT-39 and MT-42) were dry in late October 1999, and benthic macroinvertebrate samples were not collected from these monitoring stations. Benthic macroinvertebrate samples were collected from two of the three filled segments (MT-25B, MT-32), the mined stream segment (MT-45), and both the filled/residential sites (MT-40 and MT-48). The stream segment associated with MT-34B was dry, and benthic macroinvertebrate samples were not collected from this monitoring station.

Within the Island Creek watershed, the "reference" stream segments (MT-50 and MT-51) were dry during late October 1999, and benthic macroinvertebrate samples were not collected from these monitoring stations. Additionally, the stream segment associated with MT-51 was severely disturbed by the installation of a natural gas line by the local gas company. Filled monitoring stations MT-52, MT-60, and MT-57B, and the filled/residential station MT-55 stations had flowing water conditions, and benthic macroinvertebrate samples were collected from each of these sites.

3.2.3 Winter 2000

Sampling during the Winter 2000 season was implemented within the three watersheds from January 21 to January 31, 2000. Ice had to be removed from several locations to collect benthic macroinvertebrate samples. POTESTA collected benthic macroinvertebrate samples from eight of the nine sampling stations within the Mud River watershed, seven of the eight monitoring stations within the Spruce Fork watershed, and all six monitoring stations within the Island Creek watershed.

Within the Mud River watershed, benthic macroinvertebrate samples were collected from the three unmined monitoring stations (MT-02, MT-03, and MT-13), the three filled monitoring stations (MT-14, MT-15, MT-18), the filled/residential station, MT-23, and the mined site MT-01. POTESTA did not collect macroinvertevbrate samples from the drainage ditch (MT-24).

Within the Spruce Fork watershed, benthic macroinvertebrate samples were collected from both unmined stream segments (MT-39 and MT-42), two of the three filled monitoring stations (MT-25B, MT-32), the mined station (MT-45), and both the filled/residential stations (MT-40 and MT-48). The stream segment associated with MT-34B was completely frozen, and benthic macroinvertebrate samples were not collected from this monitoring station during the Winter 2000 index period.

Within the Island Creek watershed, the unmined stream segments (MT-50 and MT-51), the filled monitoring stations (MT-52, MT-60 and MT-57B), and the filled/residential (MT-55) monitoring

station had flowing water conditions, and benthic macroinvertebrate samples were collected from each of these sites during the Winter 2000 index period.

3.2.4 Spring 2000

Sampling during the Spring 2000 season was implemented within the three watersheds May 17 and 18, 2000. Within the Mud River watershed, benthic macroinvertebrate samples were collected from eight of the nine USEPA monitoring stations. POTESTA did not collect macroinvertevbrate samples from the drainage ditch (MT-24) due to inappropriate substrate for surber sampling. Within the Spruce Fork and Island Creek watersheds, benthic macroinvertebrate samples were collected from all of the USEPA monitoring stations.

3.3 Quantitative Surber Sampling

3.3.1 Sample Collection

The benthic macroinvertebrate population at each station was sampled using the quantitative Surber sampler with a 500 μm nylon mesh. The sampling procedure followed standard sampling protocols described in Standard Methods 10500B (Standard Methods, 1995). The Surber sampler was placed on the stream bottom, ensuring that the bottom frame edges of the sampler were flat against the stream bottom so that all organisms within the sampling frame would drift into the net. Cobble and large gravel were brushed thoroughly and removed from the sampling frame. The substrate was then disturbed to a depth of approximately three inches with the handle of the brush. Six Surber samples were collected at each sampling station and retained as individual replicate samples.

3.4 Sample Sorting & Identification

The samples were removed from the Surber sampler net and transferred to one-liter plastic jars with the use of a 500 μm sieve. Each sample was assigned a unique sample identification code based on the sampling site, date, and replicate number. A sampling label with the unique identification code was filled out with pencil and inserted into the jar. The unique identification code also was written on the lid of the plastic jar with a black permanent marker. The unique sample identification code also was noted in the field notebook for that specific sampling site. The samples were preserved in the field with 70 to 75 percent ethyl-alcohol. The samples were transported to the offices of POTESTA in Charleston, West Virginia, by car, by the POTESTA biologists who collected the samples.

Upon arrival at the offices of POTESTA, the samples were stored in the locked sample storage room until they were processed and identified. Samples were sorted and identified by Dr. Thomas Jones' laboratory at Alderson-Broaddus College located in Philippi, West Virginia. Some benthic macroinvertebrate samples were sorted by staff and identified to familial level by senior scientists at POTESTA and an outside consultant at Pennsylvania State University (resumes for the subcontractors have previously been provided to the USEPA). All of the samples were identified to the familial taxonomic level. Taxonomic keys used for this project included Merritt and Cummins

(1996), Wiggins (1996), and Stewart and Stark (1993). Standard quality assurance/quality control (QA/QC) measures were followed to keep track of the samples (USEA QAPP).

3.5 Data Management

3.5.1 Data Entry

The data from each sample log sheet were entered into a Microsoft ACCESS database. The database, which was developed by the West Virginia Division of Environmental Protection and the USEPA, calculated a series of bio-assessment metrics. The database was modified by POTESTA to calculate all the metrics included in this analysis. Data utilized in the analysis included only aquatic life stages of aquatic and semi-aquatic organisms. Terrestrial organisms and adults which were not aquatic were excluded. These organisms are not contributing solely to the aquatic ecosystem at the time of sampling, and their exclusion for data analysis is standard procedure. Similarly, pupae were excluded from the data set. The metrics for each sample were exported to a Microsoft EXCEL spreadsheet. Summary statistics such as mean, standard deviation, minimum value, and maximum value for each of the stream segments were calculated using Number Cruncher Statistical System (NCSS) 2000 software.

3.5.2 Statistical Analysis

The Summer and Fall 1999 datasets were not complete due to the dry conditions. These datasets were not subjected to statistical analysis. Data from the Winter and Spring 2000 sampling events were more complete and were therefore utilized in significance testing. These data are also represented graphically using Box and Whisker plots. The graphical displays allow for visualization of differences between groups and violations of assumptions. To compare different types of stream segments (unmined, filled and filled/residential) analysis of variance (ANOVA) methods were used. The calculations were performed using the general linear models (GLM) procedure on NCSS. Prior to the analysis, the data were rank transformed to reduce the effects of violations of the assumptions. Following the overall test of mean differences, the reference (unmined) mean was compared to the filled and filled/residential means using multiple comparisons based on Bonferroni adjusted t-tests. For all of the analyses, a Type I error rate of 0.05 was used.

Functional feeding groups, as described by Merrit and Cummings (1996) were determined for benthic macroinvertebrate taxa collected during the Winter and Spring 2000. The USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers - EPA 841-B-99-002 (RBP Protocol) was also referenced for functional feeding group information as necessary. Functional feeding groups included collector, filterer, scraper, shredder, predator and piercer. The feeding group designation for each identified family is indicated in Table 2. Statistical comparisons between the filled, filled/residential and unmined sites to Statistical comparison of functional groups between the filled, filled/residential and unmined sites were made using the GLM procedure on the ranked data followed by Bonferroni t-test comparisons.

3.6 Bioassessment Metrics

The metrics included herein were based on the family-level classification and have been selected by POTESTA as the most appropriate and comprehensive for use in conducting assessments of benthic macroinvertebrate communities. The metrics were selected from a larger group of widely applicable candidate metrics described in the RBP Protocol. Each of the selected metrics measured a different component of the community structure and has a different range of sensitivity to pollution/disturbance stress in the aquatic ecosystem. A description of each metric along with the expected change in response to stress is included in Table 3. The 11 metrics were:

- Total Number of Individuals (Abundance)
- Total Number of Taxa (Richness)
- Hilsenhoff Biotic Index (HBI)
- Percent Two Dominant Taxa
- Percent Chironomidae
- Total Number of EPT taxa
- Number of EPT individuals
- Percent EPT taxa
- Percent Ephemeroptera
- Percent Plecoptera
- Percent Trichoptera

3.7 Water Chemistry Analysis

USEPA personnel have collected water chemistry samples for analysis as described in the EIS document. Those data are included herein so that comparisons can be made between the treatment classes with regard to the water chemistry.

Please note that while no data included herein were disqualified due to quality assurance problems with the USEPA contract laboratories, the results of the analysis are from the "first contract laboratory" and were excluded from some of the USEPA's analysis due to perceived problems with the laboratory. Despite the potential quality issues, the data are included since they represent the only water quality information available from the study period. The data should be interpreted with caution.

Water chemistry data were analyzed using the GLM procedure on the ranked data followed by Bonferroni t-test comparisons. Statistical comparisons between the filled, filled/residential and unmined sites were made where possible. Sample size was sometimes limiting.

3.8 Habitat and Substrate Assessment

USEPA personnel have performed habitat assessments and collected substrate information at each sampling location as described in the preliminary draft EIS document. Those data are included

herein so that comparisons can be made between the treatment classes with regard to the available habitat and substrate.

Total habitat scores and measured values relating to habitat variability were analyzed using the GLM procedure on the ranked data followed by Bonferroni t-test comparisons. Statistical comparisons between the filled, filled/residential and unmined sites were made where possible.

4.0 RESULTS

The 11 bio-assessment metrics calculated for each monitoring station and season are provided in Table 3.

4.1 Summer 1999

When the benthic macroinvertebrate samples were collected in the Summer 1999 index period, six of the seven reference streams within the Mud River, Spruce Fork and Island Creek watersheds were dry or had insufficient flow to collect a sample. In contrast, all valley fill mining-influenced monitoring stations had flowing water in the summer and could be sampled. Due to the lack of reference information, no comparisons can be drawn between the reference conditions and the filled and filled/residential conditions. In addition to the obvious drought conditions, low flow conditions occurring during the highest temperatures of the year make evaluation of mining influences difficult. It appears that the presence of fills in the watershed may minimize the effects of drought conditions by supplying a more consistent flow of water to the headwater streams. However, the actual impacts that drought conditions have on stream communities are variable depending on the length and severity of the drought and the extent of refugia available for benthic macroinvertebrates to inhabit until surface conditions are more favorable. The impacts that the drought in 1999 had on the reference streams are unknown.

Data collected from the filled, filled/residential, and flowing unmined sites in the three watersheds are presented in Table 4.

4.2 Fall 1999

As occurred in the Summer 1999 sampling event, all the reference streams within the three watersheds were dry during the fall index period. One of the filled monitoring stations was dry during the Fall 1999 index period. As indicated previously, due to the lack of reference information, no comparisons can be drawn between the reference conditions and the filled and filled/residential conditions.

Data collected from the filled and filled/residential sites in the three watersheds are presented in Table 5.

4.3 Winter 2000

All 21 monitoring stations had flowing water during the Winter 2000 index period, although one monitoring station was completely frozen over and samples were not collected during the Winter 2000 sampling event. Summary statistics for each site sampled are given in Table 6. Summary statistics for each of the site types (reference, filled, or filled/residential) are included in Table 8 and the data are presented graphically in Figures 4 to 14. Boxplots are constructed using the average of the surber samples to represent one data point for each site.

Data from the three groups were compared statistically using a general linear model procedure on the ranked data. Where statistically significant differences were found between the groups, pairwise comparisons were made using t-tests with the Bonferroni adjustments. Results of the statistical analysis are presented in Table 9. As is indicated in the table, the greatest difference between the groups is in the percent mayfly metric followed by the percent EPT, percent chironomids, and percent two dominant taxa. The filled/residential sites were significantly different from the unmined sites for eight of the eleven metrics. The filled sites were significantly different from the unmined sites for two of the eleven metrics, percent mayflies and percent two dominant taxa.

The functional feeding group for each identified family was determined. Functional feeding groups are classifications that distinguish insects based on the manner in which they process nutrients. For example, a collector filter is an organism which filters nutrient material from the water column. Examining functional feeding groups may indicate to what degree a stream segment is dependent on a particular food resource (Merritt and Cummins, 1984). The function feeding groups were represented graphically for the filled, filled/residential, and unmined sites (Figure 15). The filter feeders increased in the filled and filled/residential sites with respect to the unmined sites. The collector group increased in the filled/residential sites as compared with the unmined and filled sites. Scrapers declined in the filled and filled/residential sites with respect to the unmined sites. Shredders increased slightly below the filled sites but declined in the filled/residential sites with respect to the unmined sites. Predators were similarly represented in the filled and unmined sites but decreased in the filled/residential sites.

Statistical analyses of the data indicate that collector-gatherers were significantly higher in the filled/residential sites as compared to the unmined sites (Table 10). Representatives of the piercer feeding group were also significantly reduced in the filled/residential sites as compared with the unmined category; however, there were so few piercers in the population that the differences are slight. Organisms from the scraper functional feeding group dominated the unmined sites and were significantly greater than representatives of this functional feeding group with respect to the filled sites. Of particular significance is the similarity between the unmined and filled groups with respect to shredders having 19.3 percent and 25 percent of each community comprised of these individuals, respectively. Also noteworthy is the increase in filterer-collectors in the filled and filled/residential groups, which could be attributed to increases in the organic inputs. The sources of organic enrichment would likely be domestic inputs at the filled/residential sites and the pond influence at the filled sites. Increases in collectors, particularly filter feeders, below impoundments are well documented in the literature (Allen, 2000; Stanford and Ward, 1979; Petts, 1984).

4.4 Spring 2000

All 22 monitoring stations had flowing water during the Spring 2000 index period and samples were collected from each station except MT-24, which was not sampled due to substrate limitations. Summary statistics for each site sampled are given in Table 7. Summary statistics for each of the site types (reference, filled, or filled/residential) are included in Table 11, and the data are presented graphically in Figures 16 to 26. Boxplots are constructed using the average of the surber samples to represent one data point for each site.

As with the winter index period, data from the three groups were compared statistically using a general linear model procedure on the ranked data. Where statistically significant differences were found between the groups, pairwise comparisons were made using t-tests with the Bonferroni adjustments. Results of the statistical analysis are presented in Table 12.

As shown in Table 12, the greatest difference between the groups is in the percent mayfly metric followed by the percent EPT, percent chironomids, HBI, and percent two dominant taxa. The filled/residential sites were significantly different from the unmined sites for six of the eleven metrics. The filled sites were significantly different from the unmined sites for five of the eleven metrics, including: EPT richness, percent Plecoptera, percent Ephemeroptera, and HBI.

The functional feeding group for each identified family was determined. The functional feeding groups were represented graphically for the filled, filled/residential, and unmined sites (Figure 27). As seen also in the winter data, the filter feeders increased in the filled and filled/residential sites with respect to the unmined sites. The collector group increased slightly in the filled/residential sites as compared with the unmined and filled sites. There were fewer scraper taxa in the filled and filled/residential sites with respect to the unmined sites. In contrast to the winter sampling event, shredders decreased below the filled and the filled/residential sites with respect to the unmined sites. Predators were similarly represented in the filled and unmined sites but decreased in the filled/residential sites.

Statistical analysis of the data indicates that there were no statistical differences between the unmined, filled and filled/residential groups with respect to the collector-gatherers, scrapers, or piercers (Table 13). Collector-gatherers dominated all treatments. Shredders were significantly lower in the filled and filled/residential sites than the unmined sites and filterer-collectors were significantly greater in the filled and filled/residential sites than the unmined. Predators were again significantly reduced in the filled/residential sites as compared with the unmined.

4.5 Water Chemistry Analysis

USEPA personnel have collected water chemistry samples for analysis as described in the EIS document. Those data discussed herein are included in Tables 14 and 15 with summaries showing statistical comparisons given in Tables 16 and 17.

4.6 Habitat and Substrate Assessment

Selected habitat and substrate parameters were compared with the metrics found to indicate significant differences between the unmined, filled, and filled/residential sites. The data used in the comparisons are included in Table 18 and the results of the statistical comparisons are included in Table 19.

5.0 DISCUSSION

This report is a presentation of the benthic macroinvertebrate data at the familial level. The study focused on the Mud River, Spruce Fork, and Island Creek watersheds. There was a drought during the Summer and Fall 1999 index periods.

5.1 Drought Effects

The majority of the reference streams within the three watersheds were dry during the summer and fall index periods. In contrast, valley fill stations had flowing water in the summer and all but one in the Fall 1999 index period. The extent to which the drought conditions affected the benthic communities is unknown. In response to reduced flow conditions, higher temperatures, and lower dissolved oxygen levels associated with drought conditions (Allen, 2000; Lake, 2000; Miller and Golladay, 1996), the benthic macroinvertebrate communities may experience increased predation and competition, increasing richness of opportunistic species, low abundance, and change in functional feeding group structure (Lake, 2000; Miller and Golladay, 1996). The unmined sites, which were too flow limited to be sampled, and to some extent, the filled, and filled/residential streams may have experienced all or some of these conditions related to drought conditions.

During the summer drought conditions, benthic communities in the filled and filled/residential streams were characterized by low abundance and richness in the Mud River watershed with moderate richness and abundance in the Spruce Fork and Island Creek watersheds. Filter feeding caddisflies from the family Hydropsychidae dominated benthic communities at most of the filled sites. Filled/residential sites were dominated by riffle beetles which may reflect increased algae growth due to nutrient loading from residences or decreased canopy cover in the larger, higher order streams. Stoneflies and mayflies were poorly represented in the samples; however, EPT abundance and percent EPT metrics were high due to the dominance of the Trichoptera.

Similar drought conditions were seen in the fall index period. In the Mud River watershed, the abundance increased at the filled sites. Richness also showed a slight increase as compared with the summer condition. Stoneflies were dominant at the filled site, MT-14, and increased throughout the watershed. The shredders from families Leuctridae/Capniidae and Taeniopterygidae were prevalent, and Philopotamidae, another filter feeding caddisfly, was dominant in addition to the Hydropsychidae. Chironomidae, a collector, was dominant at the filled site, MT-18. Spruce Fork and Island Creek watersheds also had increases in abundance and moderate richness. As seen in Mud River, stoneflies increased in both watersheds which also raised the EPT abundance.

Communities at sampling locations in the Spruce Fork watershed were still dominated by hydropsychids with riffle beetles, Leuctricae/Capniidae, and midges also contributing to the percent two dominant taxa metric.

Data collected during the Summer and Fall of 1999 should be interpreted carefully due to the stressful conditions of the drought and the lack of reference data for comparison. Overall, streams with valley fills are more likely to maintain flowing water conditions during dry periods. These streams are dominated by filter feeding organisms followed by shredders with scrapers, the riffle beetles, appearing in the larger more open streams.

5.2 Winter Benthic Macroinvertebrates

Benthic macroinvertebrate data collected during the winter sampling event showed differences between the unmined, filled and filled residential groups. Abundance was reduced in the unmined reference locations possibly due to the drought conditions experienced in the previous two index periods. As indicated, the effects of the fills appear to mitigate the drought and likely contributed to the higher abundance in the filled and filled/residential sites. Differences between the benthic macroinvertebrate communities in the unmined and filles sites were evident in the metrics involving the mayfly population which was decreased below the fill sites. Stoneflies were prevalent in these sites, however, indicating that water quality may not be the limiting factor for the absent mayflies, as they are both sensitive taxa. Below the filled sites, the sensitive EPT taxa still comprised an average of 50 percent of the population.

The increased variability for several metrics in the filled sites, as compared with the unmined sites, indicates that there are differences within the filled group which may limit the benthic communities at some sites but not consistently in this group. Significant differences in the filled group, which pertain to mining influences, may include the age of fill, time elapsed since fill completion, type of overburden placed in the fill, number of fills in the watershed, size of the fills, and engineering practices used in fill construction. Differences may also be due to site related conditions such as the presence of ponds or impoundments, distance from the sampling site to the impoundment, number of ponds upstream of the site, size and age of the ponds, impoundment release mechanism (surface or bottom release), general watershed characteristics (gradient, soil type, cover) and many other variables. Overall, the filled sites are only significantly different from the unmined sites with respect to the percentage of the population comprised of mayflies and the percentage of the two dominant taxa, which is not necessarily a mayfly influenced metric. Differences in both of these metrics may be attributed to the differences in food sources for the organisms in the filled sites located below the ponds associated with the fills, stream order, and differences in temperature regimes associated with the fills and the ponds.

Flowing stream systems rely on food sources typically contributed from upstream segments which are dependent on allochthonous inputs, such as leaf litter, for nutrients. The leaves are broken down by shredders which eat the leaf material and the fungi and bacteria colonizing the leaf litter. Small parts of the leaves, associated fungi and bacteria, as well as feces from the organisms contribute to the food supply of downstream collector-gatherers and filter feeding organisms. The streams with

valley fills have a sediment retention pond located typically in the most upstream reaches of the stream just below the fill area. These ponds carry out a similar function for the upstream reaches of the streams. In the ponds, biological communities are established which are dependent on algal growth, not leaf litter, as a food source. The algae and detrital material flowing from the ponds act as the food source for the downstream communities. Since this is a more continuous and less variable food supply than leaf litter, the filter feeding and gathering organisms increased below the ponds, much like they would be in the downstream reaches of rivers described by the river continuum concept. While this represents a fundamental shift in the biological community, the community created is not necessarily undesirable, it is simply different and more representative of a community located much farther downstream.

Changes in the benthic macroinvertebrate community structure below impoundments are well documented. In general, increase in density and biomass, primarily of filter feeders and collectors, and a decrease in diversity, is expected downstream of an impoundment. These changes may result from flow constancy, organic loading, temperature changes or a combination of multiple factors (Stanford and Ward, 1979; Petts, 1984; Allen, 2000). Temperature changes often play an important role in shaping community structure and vary depending on many factors including the location of the impoundment water release (surface or bottom), source of water, size and depth of the pond and retention time of the pond Kondratieff and Voshell, 1980). Summer cools and winter warms particularly impact taxa dependent on thermal cues for life cycle completion. Mayflies and stoneflies are often eliminated below impoundments (Stanford and Ward, 1979). Caddisflies and other collectors and filter feeders, as well as, amphipods, isopods, gastropods, oligochaetes, and turbellarians often increase (Stanford and Ward, 1979)

Also of interest below the fills is the presence of a shredder community very similar to the unmined reference streams. It appears that leaf litter and detritus are still available as a food source for these organisms in addition to the pond inputs. In streams where an established riparian zone is in place, stoneflies of the families Leuctridae, Capniidae, Tanaepoterygidae, and Nemouridae comprise the shredder communities in unmined areas and below the fill areas. The similar communities in the filled and unmined streams indicate that the downstream reaches of the streams are being supplied with the coarse and fine particulate organic material which are the major contribution of headwater reaches described in the river continuum theory (Vannote, et al., 1980).

During the winter sampling event, the percentage of scrapers was high in the unmined areas. This community, primarily composed of the mayfly, Ameletidae, and the beetle, Elmidae, was lower in the filled sites which may reflect the changing food source below the ponds and may be indicative of competition with the filter feeders and collectors which increased below the fills and ponds. This shift away from the scraper abundance in the filled sites contributes significantly to the decline in the mayflies below the filled sites. Because they are a sensitive taxa, a decrease in the mayfly community may appear to indicate community degradation associated with the fills and has been represented as being indicative of poor water quality due to the fills. While this may be the case, it cannot be overlooked that the entire scraper community declines in the fill sites, not just the mayflies. This includes snails, beetles (riffle beetles and waterpennys) and one caddisfly taxa. This type of shift away from a functional feeding group is most likely related to a shift in the food source.

Scouring from flooding, canopy cover from evergreen trees as opposed to deciduous trees, and many other factors could all be causing or contributing to the decline in scrapers. At this time it is not possible to discern the cause without more study.

The filled/residential sites were significantly different from the unmined sites with respect to eight of the eleven metrics and represent a different type of biological community than that which exists in the reference sites or the filled sites. Differences in the biological communities likely resulted from both the effect of fills/ponds, differences in stream order (2nd order vs. 4th order) and the increased nutrients associated with sewage inputs from residences. This is supported by the increase in filter feeders and collector gatherers with respect to the reference streams. Unlike the filled sites, the filled/residential sites did not generally show increased variability with respect to the unmined sites but consistently scored below the reference sites. While having the highest abundance, the filled/residential sites had the lowest percent EPTs and the highest percent Chironomidae. The Chironomidae are organisms more tolerant to water quality degradation including increases in metals and oxygen depletion associated with nutrient loading, such as from sewage inputs.

Most of the chironomids feed by collecting organic material from the water column. Simuliids feed by filtering nutrient rich particles from the water. Both of these tolerant organisms are prevalent in the filled/residential sites. The filter feeding caddisflies of the family Hydropsychidae were also prevalent in streams with filled/residential influences. These caddisflies are often found below ponds and below waste treatment outfalls in flowing waters. The collectors and filterers comprised almost 75 percent of the community in filled/residential stream segments indicating a significant shift in the benthic community at these sites from a scraper dominated community. The collector dominated community is dependent on organic loading from external or upstream sources. This significant shift in the community resulting from a food source change indicates that significant differences between the biological communities at the unmined and filled/residential locations are due, at least in part, to changes in organic loading between the two categories of stream.

5.3   Winter Water Chemistry

The water chemistry collected by the USEPA contributes some information to be used when discerning the causes of changes seen in the benthic communities in the filled and filled/residential sites. The parameters measured in the field include dissolved oxygen, temperature, pH and specific conductivity. The higher dissolved oxygen concentrations in the filled/residential sites support the previous discussion regarding nutrient loading in those stream segments. During the daylight hours, when photosynthesis is occurring, the dissolved oxygen is higher in nutrient rich systems. During the night time hours when there is no oxygen input from photosynthesis, there is often an oxygen sag, particularly when associated with higher temperatures, which contributes to the tolerant communities in areas with high nutrient loadings (Wetzel and Likens, 1991). Temperatures associated with the filled sites are generally higher in the winter (and likely lower in the summer) which can alter reproduction and emergence strategies of the benthic macroinvertebrates. The alkalinity is higher in the filled and filled/residential streams which will better buffer the impacts of acid precipitation in these streams. Specific conductivity, an indication of dissolved ions, is significantly higher in the filled and filled/ residential sites as compared with the unmined sites. This

is most likely caused by increased dissolution of minerals such as calcium and magnesium, that are commonly found in the sandstone and shales disturbed by mining activity. Increased surface area of fragmented rock and exposure to the elements increases weathering rates, resulting in higher concentrations of alkaline or basic ions in the runoff. This tends to be the case regardless of whether the rock material remains on top of the mined area or placed in fills.

In the Winter 2000 data, only 14 of the 33 water chemistry parameters measured by the USEPA had sufficient sample sizes for statistical comparisons of all three groups. Of these parameters, all but three were significantly different in the unmined as compared to the filled and eight were significantly different between the unmined and the filled/residential. For three of the parameters, sufficient data were available to statistically compare the only the unmined and filled sites. Sample sizes of filled/residential sites were insufficient for statistical comparisons. Of these three parameters, selenium, antimony and lead, all three were found to be significantly higher in the filled sites as compared to the unmined. The alkalinity of the unmined streams was extremely low, averaging only 13.31 mg/l CaCO₃. The filled and filled/residential sites had significantly higher buffering capacity than the unmined sites which is a significant benefit to the aquatic life in the streams. While the pH of the unmined streams was in the six to eight standard unit range (significantly lower than the filled and filled/residential sites), due to the reduced stream buffering capacity, acidic precipitation could cause excursions of the pH below the acceptable levels. Similarly, calcium and magnesium, which make up total hardness, were both higher in the filled and filled/residential streams. Hardness mitigates metals toxicity to aquatic organisms and may be important because metals, like selenium and lead, were present in all stream types.

The levels of other ions, such as chloride, nitrate, sodium and potassium, were statistically significantly elevated. However, the low levels overall likely have no biological significance. Sulfate, which is a component of rock that dissolves and leaches into the water, is significantly higher in the filled and filled/residential sites as compared with the unmined. This is likely a significant contributor to the high conductivity measured in the field.

Parameters such as iron and manganese, which are typically associated with the mining activity, were elevated in samples collected at the filled and filled/residential sites with respect to the unmined sites. However, all the samples were well below their associated water quality criteria and not in the range of causing biological impairment. Aluminum met the acute water quality criteria. There was insufficient data on these three metals for comparisons between the treatment groups.

5.4   Winter Habitat

The sites were scored using the USEPA rapid bioassessment procedures habitat analysis metrics in addition to substrate measurements. There were few differences between the habitat and substrates at the unmined, filled and filled/residential sites. The filled/residential sites tended to be from higher order streams which may explain some differences in the communities at those sites. This may also indicate that the reference streams used in this study are not appropriate to represent expected communities at the filled/residential sites. The only significant difference in habitat characteristics

between the unmined sites and the filled sites was greater stream channel alteration in the filled sites. This metric was also significantly different in the filled/residential sites.

5.5   Spring Benthic Macroinvertebrates

As in the winter sampling event, differences are seen between the unmined, filled, and filled/residential sites. Abundance was still lower in the reference streams as compared to the filled and filled/residential streams. This may result from the previous summer's drought conditions or reflect differences in food supply or other variables between the treatment groups. The EPT abundance was similar between the filled and unmined streams but higher in the filled/residential streams, which indicates the increase in the filter feeding caddisflies as described in the winter sampling event. The percentage of EPT organisms decreased slightly in the filled sites with respect to the unmined sites resulting from a decrease in percent stoneflies. The percent mayflies increased slightly. Five of the eleven metrics were significantly different in the filled treatment with respect to the unmined conditions. These metrics were primarily those associated with the EPT taxa and the HBI. Overall, variability increased in the filled streams with respect to the unmined streams. Again this indicates that while the communities at some sites may be different from the reference condition, this is not true of all the filled sites. The percentage of EPT individuals in the unmined streams changed very little from the winter sampling event while the same metric dropped 10 percent in the filled sites. This trend was mirrored in the percent plecoptera metric where there were 19 and 21 percent stoneflies in the reference streams (winter and spring, respectively) and 27 and 11 percent stoneflies in the filled streams (winter and spring, respectively). Caddisflies also decreased in both populations, and the mayflies increased in both populations. The significant difference in the EPT related metrics results from the significant differences in the stoneflies. The decline in stonefly numbers between the two sampling events perhaps results from the emergence of stoneflies in filled sites earlier than their counterparts in the reference streams due to the more consistent temperatures in the filled streams. This is supported by the substantial decrease in the shredder population in the filled sites with respect to the unmined sites. The HBI increased in both the unmined and the filled sites with the loss of the sensitive Plecoptera taxa probably contributing to the significant difference between the treatments. This is supported by the fact that the percentage of Chironomidae did not increase in either the filled or the unmined sites, which would have indicated a shift toward a more tolerant population.

While the EPT richness was significantly reduced in the filled/residential sites, the percentage of sensitive EPT taxa increased in the spring sampling event with respect to the winter event. This 23 percent increase in EPT taxa is directly attributable to a 22 percent increase in ephemeroptera. The increase is primarily due to the increase in the collector/gatherer mayflies of the family Baetidae. The increases in collector/gatherer organisms, particularly baetids, are also seen in the unmined and filled treatments and perhaps are occurring in response to the decreased scraper community in the spring when leaf cover shades the streams. This effect is pronounced in the filled and filled/residential sites due to increasing production in the ponds with increasing temperatures which provides a food supply for the collectors greater than that what would occur in typical headwater streams.

The filled/residential sites were significantly different than the unmined sites for six of the eleven metrics measured. In the winter sampling event, there were eight metrics significantly different with the overall abundance and the EPT abundance being more similar in the spring event. The increased EPT abundance indicates the previously mentioned baetid increases in the filled/residential sites. Like the filled sites, the filled/residential sites also had increases in the collector/gatherer and filterer functional feeding groups and a decrease in the scraper component of the community.

5.6   Spring Water Chemistry

In the Spring 2000 sampling event, 18 of the 35 water chemistry parameters measured by the EPA had sufficient sample sizes for statistical comparisons. Of these parameters, all but four were significantly different in the unmined sites as compared to the filled sites, and ten were significantly different between the unmined and the filled/residential. Field chemistry analysis was similar to the winter sampling event with conductivity and pH significantly higher in the filled and filled/residential sites as compared with the unmined sites. The higher temperatures and dissolved oxygen in the filled and filled/residential sites that was evident during the colder winter months was not apparent in the spring season.

The water chemistry parameters with sufficient sample sizes for statistical comparisons were slightly different in Spring 2000 from the Winter 2000 sampling event. Parameters measured in the winter showed similar trends to the previous sampling event with alkalinity and hardness related parameters highest in the filled sites. Total organic carbon was significantly higher in the filled sites again indicating a food source for aquatic organisms. Other ions, such as chloride, nitrate, sodium and potassium, were statistically significantly elevated; however, the levels are so low overall that they likely have no biological significance. Sulfate, was again elevated in the filled and filled/residential sites.

Parameters measured in the Spring 2001 sampling event that were not measured in the previous sampling event included: dissolved organic carbon, total iron, total dissolved solids and total suspended solids. Like total organic carbon, dissolved organic carbon was also significantly higher in the filled sites as compared with the unmined sites. Total suspended solids was similar among the three treatments. The average iron concentration was higher in the filled and filled residential sites, although not significantly higher. None of the average iron concentrations in either treatment approached the water quality standard for iron, so it is unlikely that this parameter will have any biological effects.

6.0   CONCLUSIONS

Changes were seen in both the benthic macroinvertebrate community and water chemistry between the unmined streams and filled and filled/residential reaches. Differences between the unmined streams and the filled streams may be related to differences in temperature regimes (and therefore emergence times), the presence of ponds (additional food source), and water chemistry differences between the treatments. Differences in stream order may also contribute to the difference between the communities at the unmined, filled and filled/residential sites. Different biological communities would be expected in a first or second order stream as compared to a third, fourth or even fifth order stream. One interesting finding is that while the most significant biological impairment was indicated in the filled/residential sites with respect to the unmined sites, the most significant changes in water chemistry, with respect to the reference sites, were seen in the filled sites. This indicates that the significant changes in the communities at the filled/residential sites (and possibly the filled sites) results from some variable other than water chemistry parameters alone.

Much information has been published on the effects of mining on benthic macroinvertebrate community structure. Among the most significant and easily observable impacts is a reduction in the sensitive EPT taxa (Beltman, et al, 1999), particularly mayflies and stoneflies which would be accompanied by a shift toward a more tolerant community. In recent years, several authors have further reported that some stoneflies were not only present but dominant in mining influenced streams where mayflies were reduced (Carlisle & Clements, 1999). While mining related impacts are often tied to metals, it is not always evident whether other factors such as sedimentation, pH, and other dissolved ions, such as sulfate, are also involved in community structure changes. The current study also indicates that changes in community structure may result from the presence of ponds which provide a different food source. All of these potential changes are related to mining in general, not necessarily to the practice of valley fill construction. Of the changes in both the water chemistry and biological communities which are described in this report, none can be attributed to the fill specifically and all potentially result from coal mining, road construction or residential development. Additionally, the same changes in both water chemistry and biological communities result from large scale development projects, and ore extraction and processing operations (ore and gold extraction, steel mills, smelters).

Neither the changes in the biological community, nor the changes in the water chemistry in the filled sites appear to have significant adverse impacts on the stream function with respect to downstream segments. The most significant changes in stream biological community appear to be the shift in the functional feeding groups toward more filter feeding organisms. This typically occurs in streams whenever ponds, dams or municipal discharges are present. The increased abundance in these sites, which likely results from the increased food sources, indicates that sufficient food is available to support a benthic community at these locations and downstream. Also, the shredder community is not reduced at the filled sites, so it can be concluded that downstream communities should be receiving a particulate organic material from these more upstream segments. While the benthic communities at the sites associated with valley fills may have a reduced mayfly population, they still support an abundant population with representatives of all the functional feeding groups, and stream function does not appear compromised at these sites.

From the data contained herein, it is not possible to discern any in-stream effects specifically attributable to valley fills as distinguished from other mining practices or other disturbances such as development, road construction, and ore extraction. Additionally, more information is necessary to identify factors which contribute to the variability in the benthic community and the water quality at the valley fill influences sites.
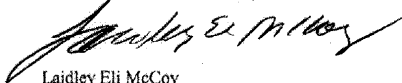
7.0 CLOSING

Potesta & Associates, Inc. has prepared this report describing the activities associated with the quantitative benthic macroinvertebrate surveys that were conducted in conjunction with the USEPA MTR/VF-EIS study on the Mud River, Spruce Fork and Island Creek watersheds during the Summer 1999, Fall 1999, and Winter 2000 sampling events. This report was prepared for the exclusive use of the client, Arch Coal, Inc. The survey sampling was conducted in accordance with generally accepted environmental practices and guidelines.
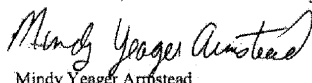
The intent of the report is to document field activities and present field observations and associated data analysis based upon our experience and professional judgement. Conclusions regarding the assessed condition(s) of the stream(s) do not necessarily represent a warranty that all segments of the stream(s) are of the same quality. Specific conditions may not be observable or readily interpreted from available information, but may become evident at a later date.

Respectfully Submitted,

POTESTA & ASSOCIATES, INC.

Laidley Eli McCoy
Vice President, Environmental Consulting

Mindy Yeager Armstead
Senior Scientist

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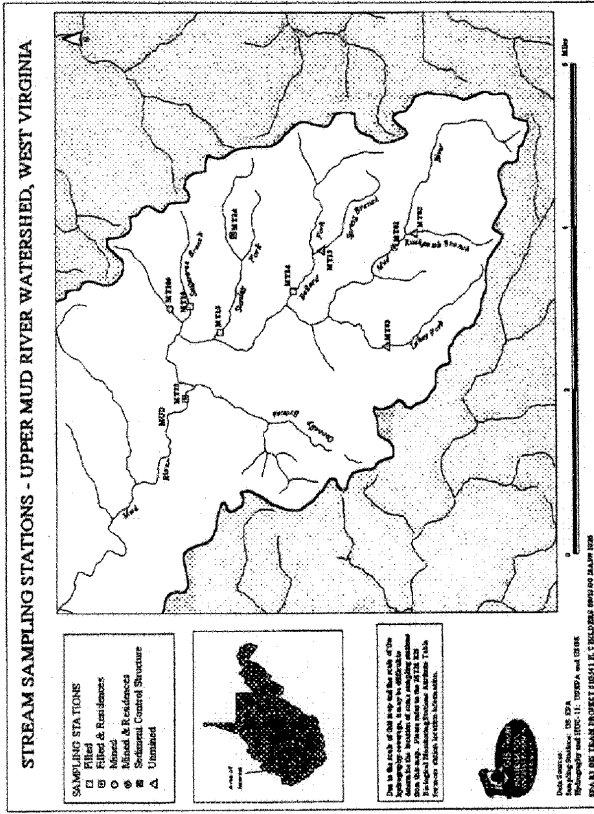
Figure 1. Stream Sampling Stations - Upper Mud River Watershed, West Virginia

Reprinted from "Mountaintop Mining/Valley Fills in Appalachia Draft Programmatic Environmental Impact Statement". US EPA June 2003
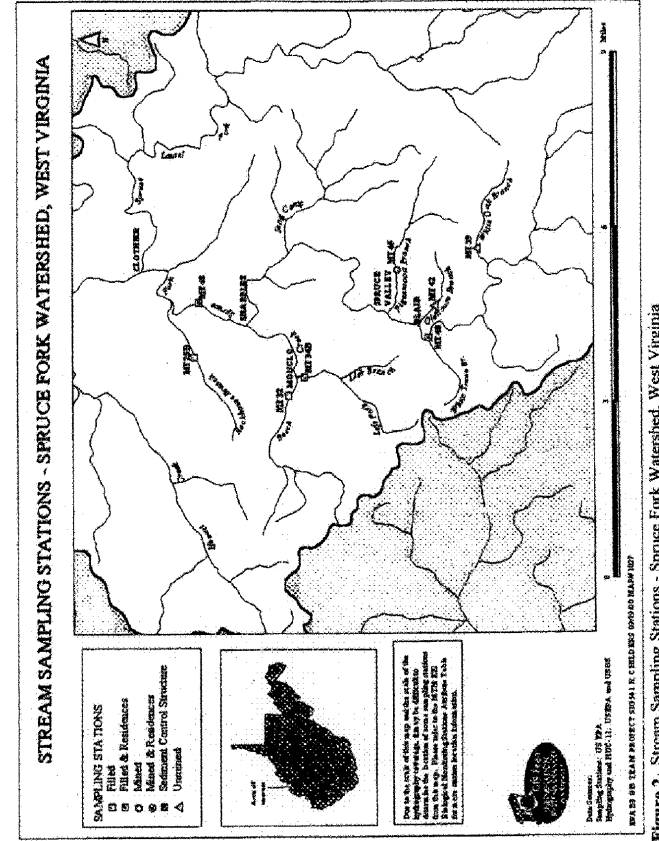
Figure 2. Stream Sampling Stations - Spruce Fork Watershed, West Virginia

Reprinted from "Mountaintop Mining/Valley Fills in Appalachia Draft Programmatic Environmental Impact Statement". US EPA June 2003

STREAM SAMPLING STATIONS - ISLAND CREEK WATERSHED, WEST VIRGINIA

SAMPLING STATIONS
☐ Filled
▣ Filled & Residences
● Mined
◉ Mined & Residences
▣ Sediment Control Structure
△ Unmined

Figure 3 . Stream Sampling Stations - Island Creek Watershed, West Virginia
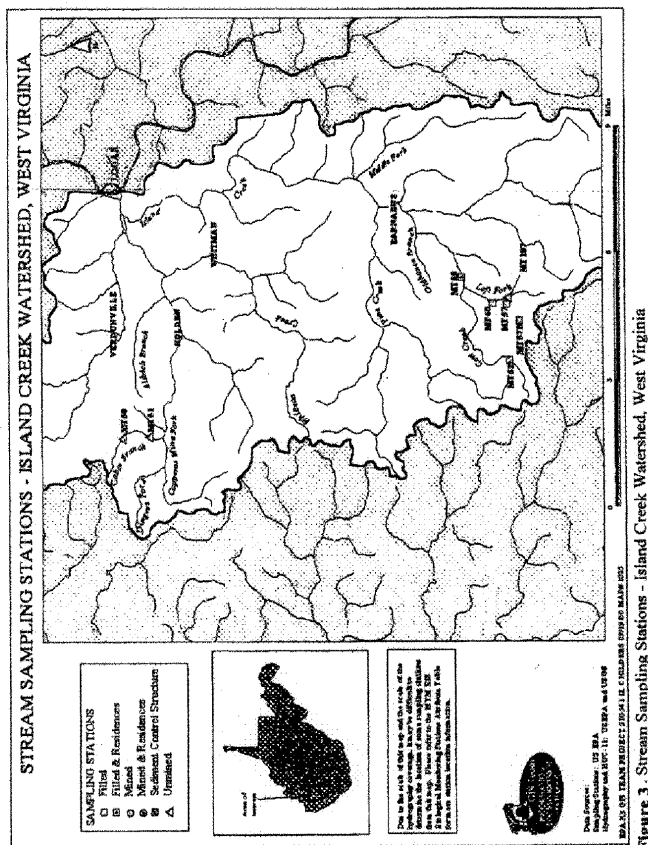
Reprinted from "Mountaintop Mining/Valley Fills in Appalachia Draft Programmatic Environmental Impact Statement". US EPA June 2003

Figures 4 – 14. Box plots of the metrics for benthic macroinvertebrate communities at Unmined, Filled and Filled/Residential sites in the Mud River, Spruce Fork and Island Creek watersheds during the Winter 2000 sampling event.

Figure 4

Figure 5

Figure 6

Box Plot

Figure 6 Box Plot — Perizdom vs Treatment (Filled, Filled/Resident, Untreated)

Figure 7

Box Plot

Figure 7 Box Plot — EPT_Abd_ vs Treatment (Filled, Filled/Resident, Untreated)

Figure 8

Box Plot

Figure 8 Box Plot — EPT_Rich vs Treatment (Filled, Filled/Resident, Untreated)

Figure 9

Box Plot

Figure 9 Box Plot — perEPT vs Treatment (Filled, Filled/Resident, Untreated)

Figure 10

Box Plot

perP vs Treatment (Fixed, Fixed/Residence, Untreated)

Figure 11

Box Plot

perE vs Treatment (Fixed, Fixed/Residence, Untreated)

Figure 12

Box Plot

perT vs Treatment (Fixed, Fixed/Residence, Untreated)

Figure 13

Box Plot

perchiros vs Treatment (Fixed, Fixed/Residence, Untreated)

Figure 14

Box Plot

Figure 15. Functional feeding groups represented at the Unmined. Filled and Filled/residential sites from the Winter 2000 sampling event.

Winter 2000
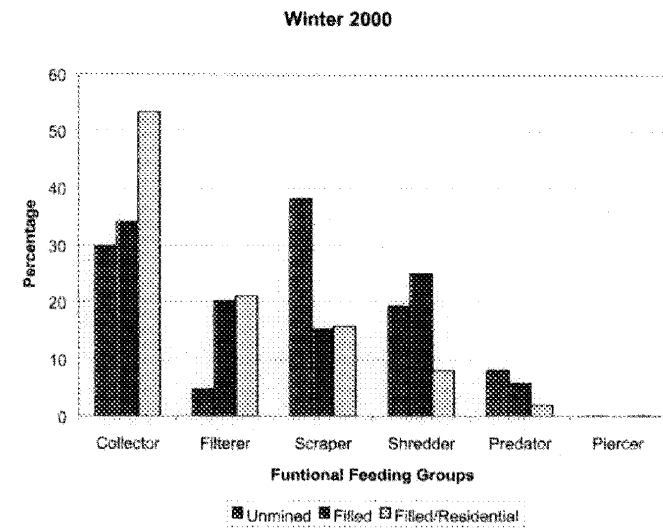
Figures 16 – 26. Box plots of the metrics for benthic macroinvertebrate communities at Unmined, Filled and Filled/Residential sites in the Mud River, Spruce Fork and Island Creek watersheds during the Spring 2000 sampling event.

Figure 16
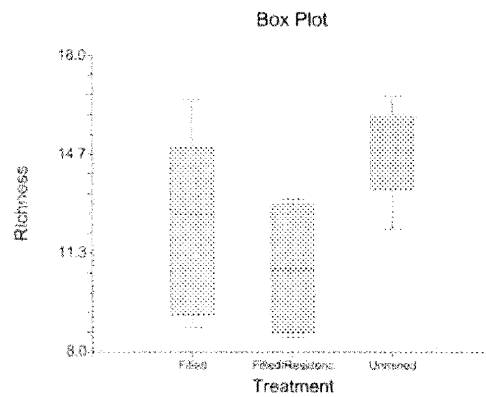
Box Plot

Figure 17

Box Plot

Figure 18

Box Plot

Figure 19

Box Plot

Figure 20

Box Plot

Figure 21

Box Plot

Figure 22

Box Plot

Figure 23

Box Plot

Figure 24

Box Plot

X__T

Treatment

Figure 25

Box Plot

X__chiros

Treatment
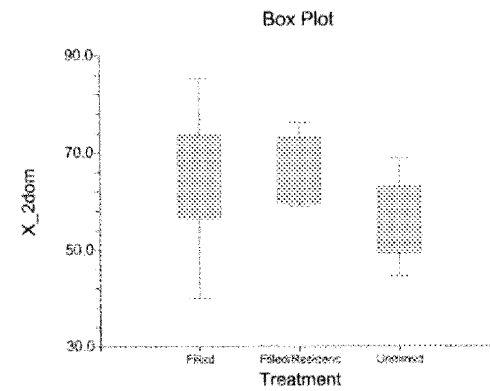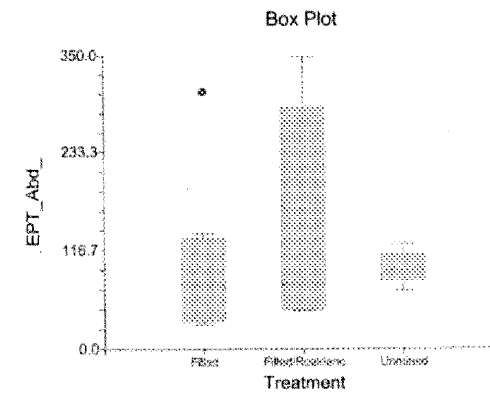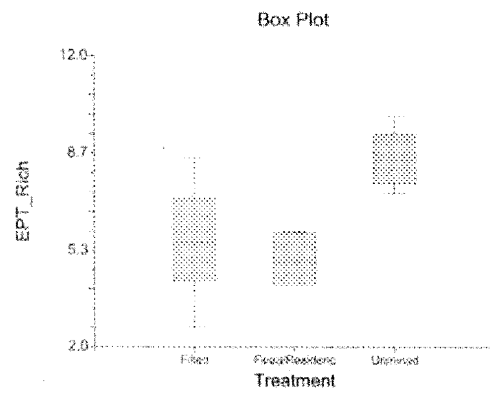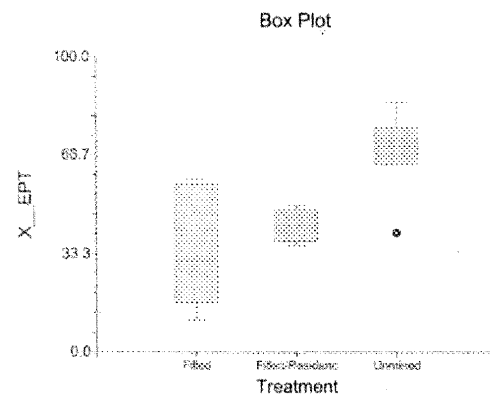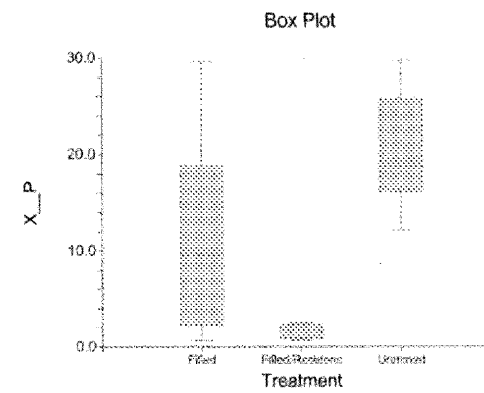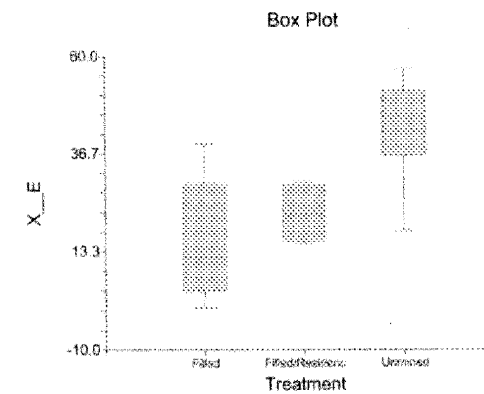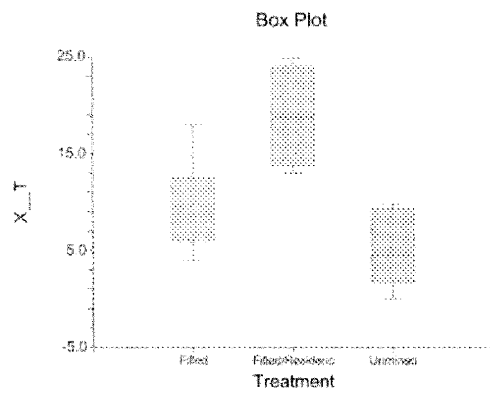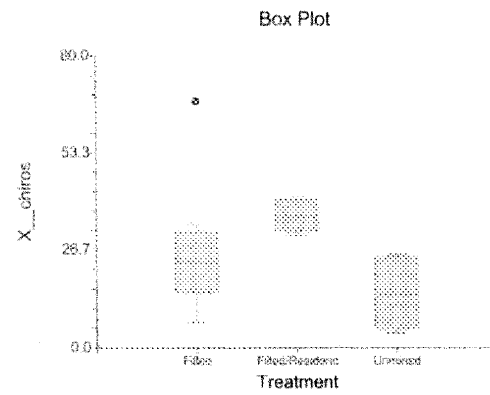
TABLE 1

TABLE 1

Monitoring Sites within the Mud River, Spruce Fork, and Island Creek Watersheds

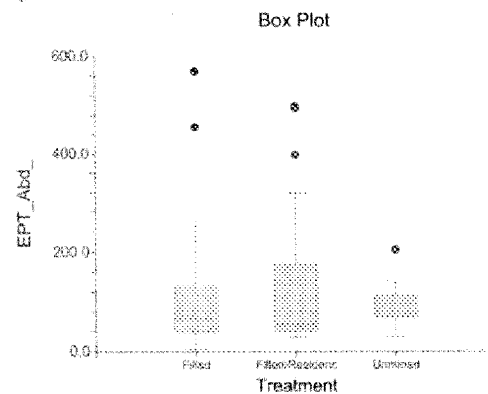|	Station Code and Name	Station Description
Unmined	MT-02 Rushpatch Branch	A second order stream, is located approximately 500 feet upstream of confluence with the Mud River.
Unmined	MT-03 Lukey Fork	A second order stream, is located approximately one mile upstream of confluence with the Mud River.
Unmined	MT-13 Spring Branch of Ballard Fork	A first order stream, is located approximately 585 feet upstream of confluence with Ballard fork.
Filled	MT-14 Ballard Fork	A second order stream, is located approximately 900 feet upstream of confluence with Mud River.
Filled	MT-15 Stanley Fork	A third order stream, is located approximately 700 feet upstream of confluence with Mud River.
Filled	MT-18 Sugartree Branch	A second order stream, is located approximately 2000 feet upstream of confluence with Mud River.
Filled/ residential	MT-23 Mud River	A fourth order stream, is located approximately 1300 feet downstream of confluence with Connelly Branch.
Not included in assessment	MT-24 Stanley	This stream segment is a sediment control structure located in the Stanley Fork Drainage.
Filled	MT-25-B Rockhouse Branch	A second order stream, is located approximately 1.2 miles upstream of the confluence with Spruce Fork.
Filled	MT-32 Beech Creek	A third order stream, is located approximately 1.9 miles upstream of the confluence with Spruce Fork.
Filled	MT-34-B Left Fork of Beech Creek	A first order stream, is located approximately 900 feet upstream of confluence with Beech Creek.
Unmined	MT-39 White Oak Branch	A second order stream, is located approximately 2000 feet upstream of confluence with Spruce Fork.
Filled/ residential	MT-40 Spruce Fork	A fourth order stream, is located in Blair, directly upstream of confluence with White Trace Branch. Site is downstream of 9 valley fills, including 2 refuse fills.
Unmined	MT-42 Oldhouse Branch	A first order stream, is located approximately 2400 feet upstream of confluence with Spruce Fork.
Mined	MT-45 Pigeonroost Branch	A third order stream, is located approximately 4500 feet upstream of confluence with Spruce Fork.

TABLE 1 (Continued)

Monitoring Sites within the Mud River, Spruce Fork, and Island Creek Watersheds

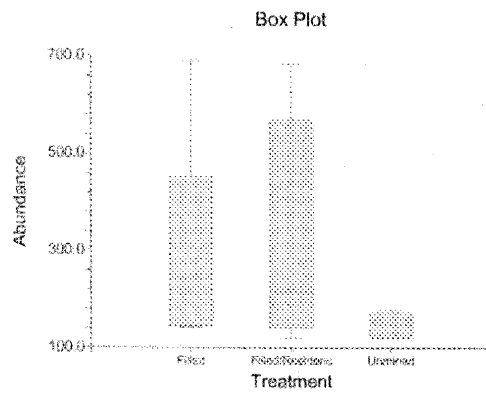|	Station Code and Name	Station Description
Filled/ residential	MT-48 Spruce Fork	A fifth order stream, is located approximately 5100 feet downstream of confluence with Beech Creek.
Unmined	MT-50 Cabin Branch	A second order stream, is located approximately 650 feet upstream of confluence with Jack's Fork.
Unmined	MT-51 Cabin Branch	A second order stream, is located approximately 1800 feet upstream of confluence with Copperas Mine Fork.
Filled	MT-52 Cow Creek	A first order stream, is located approximately three miles upstream of confluence with Left Fork.
Filled/ residential	MT-55 Cow Creek	A third order stream, is located approximately 1000 feet downstream of confluence with Left Fork.
Filled	MT-57-B Hall Fork	A first order stream, is located approximately 3600 feet upstream of confluence with Left Fork.
Filled	MT-60 Left Fork	A second order stream, is located approximately 5000 feet upstream of the confluence with Cow Creek.

TABLE 2

Benthic macroinvertebrate samples collected within the Mud River, Spruce Fork, and Island Creek Watersheds on the four sampling dates.

Station		Summer 1999	Fall 1999	Winter 2000	Spring 2000
MT-02	Unmined	NS	NS	S	S
MT-03	Unmined	NS	NS	S	S
MT-13	Unmined	NS	NS	S	S
MT-14	Filled	S	S	S	S
MT-15	Filled	S	S	S	S
MT-18	Filled	S	S	S	S
MT-23	Filled/ residential	S	S	S	S
MT-24	Sediment structure	NS	NS	NS	NS
MT-25-B	Filled	S	S	S	S
MT-32	Filled	S	S	S	S
MT-34-B	Filled	S	NS	NS	S
MT-39	Unmined	NS	NS	S	S
MT-40	Filled/ residential	S	S	S	S
MT-42	Unmined	S	NS	S	S
MT-45	Mined	S	S	S	S
MT-48	Filled/ residential	S	S	S	S
MT-50	Unmined	NS	NS	S	S
MT-51	Unmined	NS	NS*	S	S
MT-52	Filled	S	S	S	S
MT-55	Filled/ residential	S	S	S	S
MT-57-B	Filled	S	S	S	S
MT-60	Filled	S	S	S	S

S = Sampled
NS=Not Sampled

TABLE 2

TABLE 3

Benthic macroinvertebrate samples collected within the Mud River, Spruce Fork, and Island Creek Watersheds on the four sampling dates.

Metric	Description and response to stress
Total Abundance	The total number of individuals, or total abundance, characterizes the number of individuals present within the sample. This number should decrease in response to increasing perturbation (i.e., disturbance) in the stream ecosystem. However, certain individuals may increase in response to selected types of disturbance (e.g. filter feeding organisms in response to sewage pollution).
Taxa Richness	The total number of taxa, or taxa richness, characterizes the diversity of taxa present within the sample. The number of taxa should decrease in response to increasing perturbation in the stream ecosystem.
Hilsenhoff Biotic Index (HBI)	The HBI characterizes the tolerance/intolerance of the benthic macroinvertebrate community. The HBI weights each taxon in the sample by the proportion of individuals and the taxon's tolerance value. Tolerance values are assigned to each taxon on a scale of 0 to 10, with 0 identifying the least tolerant (most sensitive) organisms, and 10 identifying the most tolerant (least sensitive) organisms (USEPA 1999). The HBI is expected to increase in response to increased perturbation within the aquatic ecosystem.
Percent Two Dominant Taxa	The percent two dominant taxa metric characterizes the percentage of the two most abundant taxa in the sample. It is expected to increase in response to increased perturbation within the aquatic ecosystem.
Percent Chironomidae	The percent Chironomidae metric characterizes the percentage of midge taxa present in the sample. It is expected to increase in response to increased perturbation within the aquatic ecosystem.
EPT Richness	The total number of EPT taxa, EPT richness, characterizes the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa present in the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.

TABLE 3

Metric	Description and response to stress
EPT Abundance	The number of EPT individuals, EPT abundance, characterizes the number of sensitive EPT taxa within the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.
Percent EPT Individuals	The percent EPT individuals characterizes the percent of sensitive EPT organisms present in the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.
Percent Ephemeroptera	The percent Ephemeroptera characterizes the percent of mayflies present in the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.
Percent Plecoptera	The percent Plecoptera characterizes the percent of stoneflies present in the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.
Percent Trichoptera	The percent Trichoptera characterizes the percent of caddisflies present in the sample. It is expected to decrease in response to increased perturbation within the aquatic ecosystem.

TABLE 4a

Table 4. Summary of benthic macroinvertebrate analysis from samples collected in the Summer of 1999.

4.a. Mud River - Summer 1999				
	MT-14 Filled	MT-15 Filled	MT-18 Filled	MT-23 Filled/Resid
Number (Abundance)				
Avg	60.33	23.17	71.50	173.67
SD	59.59	12.70	58.58	114.85
Max	146.00	47.00	172.00	348.00
Min	8.00	12.00	17.00	17.00
Taxa (Richness)				
Avg	7.67	5.00	8.00	10.50
SD	2.34	1.26	1.87	2.88
Max	11.00	6.00	11.00	14.00
Min	5.00	3.00	6.00	6.00
Percent 2 Dominant Taxa				
Avg	70.87	77.50	65.00	74.47
SD	7.04	13.38	17.15	8.65
Max	81.51	95.00	86.63	90.52
Min	62.50	58.33	45.28	65.70
EPT Abundance				
Avg	34.67	15.50	51.83	23.17
SD	40.25	10.80	57.11	14.23
Max	100.00	31.00	149.00	42.00
Min	1.00	1.00	4.00	1.00
EPT Richness				
Avg	2.33	1.50	2.00	3.17
SD	1.51	0.55	0.00	1.33
Max	5.00	2.00	2.00	5.00
Min	1.00	1.00	2.00	1.00
Percent EPT				
Avg	45.10	60.93	56.25	14.56
SD	20.72	29.61	26.29	9.49
Max	68.49	87.50	86.63	29.00
Min	12.50	8.33	23.53	5.88
Percent Plecoptera				
Avg	1.67	0.00	0.00	0.00
SD	2.02	0.00	0.00	0.00
Max	4.76	0.00	0.00	0.00
Min	0.00	0.00	0.00	0.00
Percent Ephemeroptera				
Avg	0.00	0.00	0.00	0.00
SD	0.00	0.00	0.00	0.00
Max	0.00	0.00	0.00	0.00
Min	0.00	0.00	0.00	0.00
Percent Trichoptera				
Avg	43.43	60.93	56.25	14.56
SD	20.82	29.61	26.29	9.49
Max	66.44	87.50	86.63	29.00
Min	12.50	8.33	23.53	5.88
Percent Chironomidae				
Avg	5.52	2.78	13.99	11.77
SD	5.48	6.80	10.04	6.40
Max	14.29	16.67	26.92	19.68
Min	0.00	0.00	3.49	2.59
HBI				
Avg	5.04	4.67	5.43	4.51
SD	0.32	0.38	1.02	0.31
Max	5.50	4.91	6.76	4.95
Min	4.52	3.92	4.32	4.14

TABLE 4b

4.b. Spruce Fork - Summer 1999					
	MT- 25B	MT-32	MT-34B	MT-40	MT-42	MT-48
	Filled	Filled	Filled	Filled/Resid	Unmined	Filled/Resid
Number (Abundance)						
Avg	266.17	564.50	21.33	398.33	83.83	378.17
SD	115.77	316.95	4.72	174.64	14.19	72.13
Max	496.00	1146.00	28.00	534.00	102.00	457.00
Min	164.00	230.00	14.00	103.00	65.00	288.00
Taxa (Richness)						
Avg	11.00	13.67	5.83	11.33	15.67	16.00
SD	2.28	3.44	1.47	2.07	2.80	3.58
Max	13.00	20.00	8.00	14.00	21.00	20.00
Min	8.00	11.00	4.00	9.00	13.00	11.00
Percent 2 Dominant Taxa						
Avg	78.41	86.34	75.78	74.21	40.66	76.67
SD	6.42	3.26	10.67	10.20	5.28	9.48
Max	90.32	90.84	89.47	89.29	50.53	89.02
Min	73.15	83.44	61.90	63.30	35.38	67.83
EPT Abundance						
Avg	177.83	31.83	2.83	213.67	33.17	86.17
SD	116.73	11.82	3.25	88.30	10.72	65.80
Max	402.00	49.00	9.00	322.00	49.00	209.00
Min	69.00	17.00	0.00	56.00	22.00	14.00
EPT Richness						
Avg	3.00	3.17	1.17	4.67	6.00	4.67
SD	0.89	1.94	0.75	1.21	1.79	1.21
Max	4.00	6.00	2.00	6.00	9.00	6.00
Min	2.00	1.00	0.00	3.00	4.00	3.00
Percent EPT						
Avg	58.16	6.14	14.33	56.36	40.00	22.34
SD	13.54	1.48	17.12	17.08	11.97	13.88
Max	81.05	8.22	47.37	85.71	56.98	45.73
Min	42.07	4.28	0.00	37.40	23.16	3.41
Percent Plecoptera						
Avg	0.20	0.03	0.60	0.00	22.32	0.00
SD	0.50	0.08	1.46	0.00	8.17	0.00
Max	1.22	0.20	3.57	0.00	29.23	0.00
Min	0.00	0.00	0.00	0.00	7.37	0.00
Percent Ephemeroptera						
Avg	0.15	0.37	0.00	7.65	9.92	1.06
SD	0.37	0.44	0.00	4.68	4.19	0.98
Max	0.92	1.07	0.00	15.53	15.79	2.63
Min	0.00	0.00	0.00	2.86	3.08	0.00
Percent Trichoptera						
Avg	57.81	5.74	13.74	48.71	7.76	21.28
SD	13.83	1.67	17.62	19.98	5.94	13.22
Max	81.05	8.22	47.37	82.86	16.28	43.11
Min	40.85	4.10	0.00	29.07	0.00	2.93
Percent Chironomidae						
Avg	14.82	2.72	0.79	26.17	18.15	5.28
SD	10.60	0.60	1.94	11.57	14.60	3.25
Max	27.56	3.47	4.76	36.89	40.00	8.57
Min	0.60	1.83	0.00	6.43	3.08	0.98
HBI						
Avg	5.73	4.32	7.70	5.21	3.89	4.49
SD	0.21	0.18	0.83	0.12	0.66	0.15
Max	6.08	4.58	8.92	5.40	5.05	4.72
Min	5.49	4.14	6.75	5.10	3.23	4.29

TABLE 4c

4.c. Island Creek - Summer 1999	MT-52	MT-55	MT-57B	MT-60
	Filled	Filled/Resid	Filled	Filled
Number (Abundance)				
Avg	99.00	528.17	64.67	110.50
SD	61.08	158.17	72.01	44.23
Max	214.00	745.00	195.00	191.00
Min	36.00	313.00	1.00	67.00
Taxa (Richness)				
Avg	11.67	15.17	10.17	15.50
SD	2.80	2.86	7.63	1.87
Max	17.00	19.00	20.00	17.00
Min	9.00	11.00	1.00	12.00
Percent 2 Dominant Taxa				
Avg	63.60	74.36	68.29	57.32
SD	9.13	6.01	17.25	12.15
Max	74.70	81.56	100.00	71.43
Min	47.22	67.59	54.46	38.96
EPT Abundance				
Avg	53.50	211.33	29.33	60.00
SD	29.49	83.97	36.49	25.26
Max	104.00	301.00	89.00	92.00
Min	19.00	100.00	1.00	30.00
EPT Richness				
Avg	4.33	4.67	3.00	6.50
SD	0.82	1.03	2.45	1.22
Max	5.00	6.00	7.00	8.00
Min	3.00	3.00	1.00	5.00
Percent EPT				
Avg	54.82	39.00	48.63	53.85
SD	5.66	6.43	30.49	10.33
Max	62.86	47.78	100.00	68.07
Min	46.60	31.95	9.09	38.96
Percent Plecoptera				
Avg	9.96	0.23	1.00	8.72
SD	5.83	0.09	1.67	6.32
Max	19.44	0.32	3.96	19.48
Min	3.33	0.13	0.00	2.09
Percent Ephemeroptera				
Avg	0.16	1.51	0.00	2.17
SD	0.38	0.52	0.00	1.28
Max	0.93	1.92	0.00	4.48
Min	0.00	0.52	0.00	0.88
Percent Trichoptera				
Avg	44.71	37.29	47.63	42.96
SD	10.24	6.32	30.30	13.99
Max	56.19	46.03	100.00	59.66
Min	33.33	29.71	9.09	18.18
Percent Chironomidae				
Avg	1.81	11.78	8.67	5.89
SD	0.66	2.80	4.27	3.26
Max	2.78	15.74	15.15	11.69
Min	1.11	7.67	3.85	2.08
HBI				
Avg	4.24	5.02	4.39	4.66
SD	0.34	0.27	0.39	0.16
Max	4.67	5.33	5.00	4.84
Min	3.77	4.66	3.91	4.47

TABLE 5a

Table 5. Summary of benthic macroinvertebrate analysis from samples collected in the Fall of 1999.

	5.a. Mud River - Fall 1999			
	MT-14 Filled	MT-15 Filled	MT-18 Filled	MT-23 Filled/Resid
Number (Abundance)				
Avg	503.50	79.50	130.17	155.00
SD	304.43	25.89	58.51	84.19
Max	1065.00	115.00	218.00	279.00
Min	239.00	43.00	66.00	65.00
Taxa (Richness)				
Avg	8.50	8.83	10.33	10.00
SD	1.52	1.72	1.86	2.10
Max	10.00	11.00	12.00	14.00
Min	6.00	7.00	7.00	8.00
Percent 2 Dominant Taxa				
Avg	92.83	60.81	56.04	60.11
SD	3.72	10.04	6.80	10.72
Max	98.03	72.55	65.31	72.55
Min	87.65	48.10	49.04	40.91
EPT Abundance				
Avg	481.67	48.67	65.17	90.17
SD	305.03	20.03	49.45	63.08
Max	1046.00	73.00	144.00	165.00
Min	220.00	16.00	15.00	28.00
EPT Richness				
Avg	3.00	3.67	3.17	4.00
SD	0.63	1.03	0.41	1.10
Max	4.00	5.00	4.00	6.00
Min	2.00	2.00	3.00	3.00
Percent EPT				
Avg	94.45	59.76	45.81	53.78
SD	2.97	14.89	19.56	16.80
Max	98.22	79.45	66.06	75.00
Min	0.00	0.00	0.00	0.00
Percent Plecoptera				
Avg	91.48	24.96	14.60	11.97
SD	2.44	12.84	9.09	4.53
Max	94.93	43.14	25.69	17.05
Min	87.65	6.98	2.04	3.92
Percent Ephemeroptera				
Avg	0.00	0.00	0.00	0.00
SD	0.00	0.00	0.00	0.00
Max	0.00	0.00	0.00	0.00
Min	0.00	0.00	0.00	0.00
Percent Trichoptera				
Avg	2.97	34.80	31.20	41.81
SD	1.39	12.06	11.78	15.15
Max	5.01	53.42	43.94	62.73
Min	1.21	16.67	13.27	23.53
Percent Chironomidae				
Avg	0.59	12.19	34.15	11.60
SD	0.46	11.64	15.64	3.78
Max	1.26	34.88	56.12	16.13
Min	0.00	3.48	17.89	6.36
HBI				
Avg	1.37	4.66	4.69	4.40
SD	0.13	0.86	0.75	0.32
Max	1.60	5.70	5.81	4.83
Min	1.19	3.57	3.67	3.91

TABLE 5b

5.b. Spruce Fork - Fall 1999				
	MT-25B	MT-32	MT-40	MT-45	MT-48
	Filled	Filled	Filled/Resid	Filled/Resid	Filled/Resid
Number (Abundance)					
Avg	138.67	1141.50	574.17	135.00	706.50
SD	36.79	795.27	316.66	72.09	451.44
Max	185.00	2707.00	1146.00	233.00	1530.00
Min	82.00	528.00	229.00	41.00	294.00
Taxa (Richness)					
Avg	10.33	11.67	10.50	14.17	15.83
SD	1.97	2.50	2.59	2.32	4.31
Max	14.00	15.00	15.00	17.00	21.00
Min	9.00	8.00	8.00	11.00	10.00
Percent 2 Dominant Taxa					
Avg	61.16	66.64	79.77	46.04	64.00
SD	4.58	3.31	7.19	12.86	10.24
Max	65.93	70.61	90.84	67.80	75.17
Min	53.66	63.17	69.20	29.89	48.30
EPT Abundance					
Avg	89.17	416.33	408.00	94.17	233.17
SD	27.84	519.62	295.90	62.09	231.51
Max	132.00	1443.00	982.00	172.00	671.00
Min	55.00	25.00	198.00	24.00	34.00
EPT Richness					
Avg	5.33	3.67	3.83	6.83	6.67
SD	1.21	1.63	1.17	1.60	2.16
Max	7.00	5.00	6.00	9.00	10.00
Min	4.00	1.00	3.00	5.00	4.00
Percent EPT					
Avg	64.42	28.81	68.98	65.55	27.52
SD	10.47	17.64	14.56	12.43	11.73
Max	77.86	53.31	86.46	80.49	43.86
Min	50.00	4.73	52.46	45.98	10.18
Percent Plecoptera					
Avg	27.62	10.30	0.59	30.94	7.57
SD	6.93	7.04	1.22	18.46	7.33
Max	37.80	20.17	3.08	56.10	18.30
Min	19.08	0.00	0.00	10.34	0.00
Percent Ephemeroptera					
Avg	0.36	0.23	6.14	13.15	4.63
SD	0.64	0.37	4.19	7.37	2.32
Max	1.59	0.85	13.54	25.29	8.15
Min	0.00	0.00	1.83	5.85	2.40
Percent Trichoptera					
Avg	36.44	18.28	62.25	21.47	15.32
SD	10.34	13.83	14.00	7.05	7.13
Max	49.73	33.14	83.86	31.71	21.70
Min	21.43	0.98	46.67	10.34	5.10
Percent Chironomidae					
Avg	25.27	9.22	14.62	6.85	24.03
SD	8.47	8.22	12.29	5.42	10.16
Max	40.48	19.59	33.10	17.07	36.73
Min	16.76	0.00	0.00	1.46	7.65
HBI					
Avg	4.50	4.48	4.97	3.47	4.56
SD	0.48	0.55	0.20	0.86	0.30
Max	5.32	5.55	5.10	4.49	4.92
Min	3.98	4.01	4.58	2.63	4.18

TABLE 5c

S.c. Island Creek - Fall 1999			
MT-52 Filled	**MT-55** Filled/Resid	**MT-57B** Filled	**MT-60** Filled
Number (Abundance)			
Avg 328.17	254.83	195.67	139.83
SD 141.60	128.07	128.21	86.30
Max 446.00	403.00	330.00	286.00
Min 110.00	103.00	21.00	57.00
Taxa (Richness)			
Avg 13.17	9.67	12.17	11.50
SD 3.13	1.86	2.56	1.05
Max 19.00	13.00	15.00	13.00
Min 10.00	8.00	9.00	10.00
Percent 2 Dominant Taxa			
Avg 76.20	79.48	75.66	60.78
SD 8.43	8.41	12.17	8.52
Max 88.86	91.26	85.62	73.68
Min 65.50	65.38	57.14	52.31
EPT Abundance			
Avg 249.67	129.83	165.17	94.67
SD 160.59	114.58	113.87	63.02
Max 399.00	274.00	291.00	201.00
Min 17.00	5.00	10.00	38.00
EPT Richness			
Avg 5.67	2.67	5.17	5.83
SD 1.63	1.37	1.47	1.17
Max 8.00	5.00	7.00	7.00
Min 4.00	1.00	3.00	4.00
Percent EPT			
Avg 65.73	40.55	77.64	66.68
SD 29.75	27.12	15.97	9.56
Max 92.58	70.26	89.58	75.44
Min 15.45	4.85	47.62	51.79
Percent Plecoptera			
Avg 50.93	0.93	66.42	21.48
SD 26.55	0.85	18.75	9.46
Max 82.60	2.20	83.40	37.76
Min 5.45	0.00	42.05	10.53
Percent Ephemeroptera			
Avg 0.76	0.94	0.79	1.08
SD 1.15	1.15	1.94	1.39
Max 2.73	2.75	4.76	3.57
Min 0.00	0.00	0.00	0.00
Percent Trichoptera			
Avg 14.04	38.69	10.43	44.13
SD 9.29	28.03	10.76	12.78
Max 31.61	69.23	30.68	63.16
Min 6.50	2.91	0.00	32.17
Percent Chironomidae			
Avg 3.19	31.35	8.15	8.64
SD 2.62	20.51	5.85	6.63
Max 7.27	60.19	19.05	16.96
Min 0.00	8.28	2.32	0.35
HBI			
Avg 2.59	5.18	2.25	4.07
SD 0.88	0.16	0.62	0.46
Max 4.11	5.43	3.14	4.67
Min 1.56	4.99	1.76	3.39

TABLE 6a